AMELIORATION OF SALINITY TOXICITY IN SOUTH AFRICAN CROP PLANTS WITH SPECIAL REFERENCE TO MAIZE.

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ABSTRACT

The effects of ammonium and nitrate nutrition on potassium uptake, photosynthetic gas exchange and growth responses to salinity stress (80 mM) together with the ameliorative roles of three ranges of calcium concentrations (2.5 to 12 mM, 1 to 8 mM and 0.5 to 5 mM) and one range of potassium concentrations (0.2 to 5 mM) were investigated in *Zea mays* L. var PNR 394. The ameliorative roles of two ranges of calcium concentrations (1 to 8 mM and 0.5 to 5 mM) were also investigated in salt-stressed maize grown at high (35 ^{0}C) and low (25 ^{0}C) temperature conditions in order to establish if the role of calcium in enhancing salt-tolerance in maize could be temperature-dependent. The criteria chosen to monitor salinity stress were (i) dry plant mass, (ii) plant moisture content, (iii) photosynthetic performance and (iv) nitrogen uptake.

Under both saline and non-saline conditions the potassium contents of the shoots and roots of ammonium-fed plants were significantly lower than those of nitrate-fed plants indicating the inhibition of potassium uptake by ammonium. Salinity reduced the shoot and root growth of nitrate-fed plants by 27 % and 34 % respectively, whereas shoot and root growth of ammonium-fed plants were reduced by 59 % and 60 % respectively. These results indicated that ammonium-fed plants were much more sensitive to salinity than nitrate-fed plants. Photosynthetic rates and transpiration rates of nitrate-fed plants did not show any significant responses to salinity whereas those of ammonium-fed plants were significantly reduced by salinity. The possible causes for the disparity between nitrate- and ammonium-fed plants in their response to salinity are discussed. In both nitrate- and ammonium-fed plants the shoot:root ratio of salt-stressed plants were not significantly different from those of non-salt-stressed plants indicating that salinity did not affect shoot growth more than root growth or vice versa.

Potassium contents of shoots and roots of both nitrate- and ammonium-fed plants were reduced by salinity. ^{15}N studies carried out in this research also indicated salinity disturbance of the uptake of nitrate. In both nitrate- and ammonium-fed plants the moisture contents of whole plants, shoots and roots were significantly reduced by salinity. These findings are regarded as evidence for the supposition that nutrient induced deficiency and water stress are some of the major limitations on the growth of salt-stressed plants.

When grown under low temperature (25 ^{0}C) conditions and supplied with low calcium concentrations (0,5 mM and 1 mM) salinity-stressed plants grew significantly larger than non-salt-stressed plants, a feature which was not observed under high temperature (35 ^{0}C) conditions. These results indicated a temperature-dependent calcium sparing activity of NaCl.

In all ranges of calcium concentrations (2.5 to 12 mM, 1 to 8 mM and 0.5 to 5 mM) no significant effect of calcium on photosynthetic rates, moisture contents and growth of salt-stressed plants was evident under high temperature (35 ^{0}C) or low temperature (25 ^{0}C) conditions. Calcium also did not show any ameliorative role on the uptake of nitrate in salt-stressed plants. These results indicated that the supposition that calcium plays a significant role in enhancing salt-tolerance in crop plants and that its ameliorative role could be temperature-dependent is not true for this maize variety.

Increasing the concentration of potassium in the nutrient media improved the growth of salt-stressed and non-salt-stressed maize plants fed nitrate or ammonium. This effect is ascribed to the role of potassium in nitrogen metabolism.

CHAPTER 1

INTRODUCTION

Many of the third world countries face serious water supply problems in food production, although certain of these countries have large supplies of brackish water present in underground aquifers in many drier regions (e.g. the Karoo in South Africa). With human population numbers constantly increasing and malnutrition a serious problem in these third world countries, utilizing agricultural drainage waters and ground waters for irrigation is becoming essential to meet the increasing demands for food production. However, in most cases, the salinity of these waters place potential limitations on yield, either by influencing physiological functions of the plants and/or indirectly by degrading the physiological behaviour of the soils due to an unfavourable cationic environment (Rengasamy, 1987). The agronomic problem of salinity is compounded by the relatively low salt tolerance of many of the crop plants (Maas and Hoffman, 1977). The optimum use of these brackish waters requires management procedures to be adopted that will minimize the adverse effects of salts on both plants and soils.

This research study was designed to explore plant physiological strategies for ameliorating salinity toxicity in maize plants. Maize was selected since it is one of the major agronomically important cereal crop in most third world countries (more especially in South Africa). In view of studies undertaken by other researchers, the following salinity-related features were investigated: the protective effects of different forms of nitrogen (ammonium and nitrate) against salinity toxicity, the role of different ranges of calcium concentrations in ameliorating salinity toxicity and the role of potassium in ameliorating salinity toxicity.

Effects of the different treatments on salt-stressed maize plant productivity were monitored by (i) dry mass determinations (ii) photosynthetic performance determinations using IRGA techniques and (iii) nitrogen absorption determinations using ^{15}N atomic emission spectroscopy and atomic absorption spectroscopy.

CHAPTER 2

LITERATURE REVIEW

2.1 FACTORS INVOLVED IN SALT STRESS

Three major potential limitations on the growth of plants in saline environments have been identified (Bernstein,1964; Bernstein and Hayward, 1958; Maas and Niemand, 1978) and they can be categorized as follows :-

(i) Osmotic effect or Water stress

(ii) Ion imbalance stress or induced nutrient deficiency

(iii) Specific ion toxicity.

According to the stress terminology (Levitt, 1980) the osmotic effect and the ion imbalance stress are secondary salt-induced stresses, while specific ion toxicity is a primary salt injury.

There is no concesus as to the relative importance of these detrimental stresses, and indeed various researchers differ widely in their assessments. Wyn Jones (1981) maintained that in physiological and biological terms these stresses may not be too clearly delineated and they may also be closely inter-related.

2.1.1 **Secondary salt-induced stresses.**

2.1.1.1 Osmotic stress

There is a direct and inseparable relationship between the salt and the water stress. Since the addition of salt to the water lowers its osmotic potential, the salt stress will expose the plant to secondary osmotic stress. Therefore if a plant is transferred from low to high salt media it is immediately subjected to an osmotic dehydration. This osmotic dehydration can in turn cause a decrease in osmotic and water potential of the salt-stressed tissues. The results of this will be a decrease in turgor pressure and since cell elongation is turgor-dependent and contributes considerably to vegetative growth, growth of salt-stressed plants will be inhibited. Munns and Passioura (1984) reported an increase in the osmotic pressure (indicating a reduction in osmotic potential) of the xylem sap collected from the leaf tip of barley plants

exposed to salinity levels of up to 200 mM. Since growth of these salinized plants was reduced, this reduction in growth was attributed to the reduction in osmotic potential. An increase in osmotic pressure (reduction in osmotic potential) in both growing and mature tissues of saline-stressed (100 mM NaCl) wheat seedlings was also reported by Termaat and Munns (1986). This reduction in osmotic potential was also followed by a reduction in plant growth. Walker et.al (1983) reported a reduction in water potential of citrus plants leaves exposed to salinity levels of up to 150 mM. This reduction in leaf water potential was also regarded as a cause of growth reduction of those plants.

Other evidence showing that water stress is a significant factor in the inhibition of growth by external salts comes from the work of Gaugh and Wadleigh (1944) in which they showed isosmotic concentrations of different salt solutions (NaCl, $CaCl_2$ and Na_2SO_4) to produce similar reduction in the growth of salt-sensitive glycophytes.

Crassulacean acid metabolism (CAM) is probably a metabolic adaptation to semi-arid habitats (Osmond, 1978). In *Mesembryanthenum crystallinum* a shift from conventional C_3 metabolism to CAM is induced by NaCl stress and by other treatments likely to promote water stress, which again implies that water stress is a major factor in salt toxicity (Wyn Jones 1981).

The shift from high salt-sensitivity to salt-tolerance shown by some plants under high relative humidity conditions has also been suggested as evidence indicating that water stress is a significant factor in the inhibition of growth by external salt. For example O'Learly (1975) found red kidney bean (*Phaseolus vulgaris*) plants grown at NaCl levels of up to 9800 ppm to be able to survive, flower and produce normal-sized pods, the relative humidity was maintained at the range of between 95 and 100%. Reducing the relative humidity below the above mentioned range resulted in death of those plants. When working with anion and radish crop plants Hoffman and Rawlins (1971) found high relative humidity to be able to raise the salinity level at which the yield was reduced to 50% of the non-salinized plants. Terry and Waldron (1986) also found the reduction in the growth of sugarbeet exposed to salinity levels of up to 250 mM to be brought about by salinity-induced changes in leaf water status, which could be almost completely offset by increase in relative humidity.

Though studies discussed above suggested water stress as a significant factor in the inhibition of plant growth by external salts, other studies have shown that the water status of salt-stressed plants do not play a significant role in reducing plant growth. By reducing the osmotic pressure of the saline medium, thereby raising the water potential of the saline medium to the same level as that of unsalinized medium, it is possible to show if reduction in growth of salinized plants is turgor-related (related to osmotic or water stress) or not. Using this technique Termaat et.al (1985) found that even though the water potential of the saline media was the same as that of the control, growth of salt-stressed wheat and barley plants was highly reduced as compared to that of their controls. From this study it was concluded that growth reduction in salinized plants was not related to the water status of the plants. Gale et al. (1967) also found salinity to be have no effect on the water balance of onion, bean and cotton.

2.1.1.2 Induced nutrient deficiency

The decreased growth due to salinization can also be explained by a suppression of nutrient absorption due to uptake of NaCl ions in competition with nutrient ions such as calcium, potassium, nitrate and ammonium.

Lynch and Läuchli (1985) reported a decrease in the calcium content of the shoots of two salt-stressed cultivars of barley (*Hordeum vulgaris*) grown under field conditions. Devitt et.al (1984) also found a reduction in Ca^{2+} concentration in the upper leaves of sorghum and wheat plants grown in media containing low K^+/Na^+ ratios. Grieve and Fujiyama (1987) report calcium nutrition of two rice cultivars to be highly affected by Na^+/Ca^{2+} ratio of the saline nutrient solution. In this study the laminae of the plants exhibited Ca-deficiency symptoms at high Na^+/Ca^{2+} ratios in the nutrient medium. This Ca-deficiency was found to be due to Na-induced inhibition of Ca uptake.

When investigating the short-term influxes of K^+ and Na^+ ions in salt-stressed cotton roots, Cramer et al (1987) found a reduction in K^+ influx with increasing salinity. Silberbush and Ben-Asher (1987) found the main effect of salinity on nutrient uptake in cotton seedlings to be a reduction of K^+ influx, causing a decrease in the K^+/Na^+ ratio. Hajibagheri et al (1987) reported a reduction in K^+ accumulation by roots and shoots of maize seedlings exposed to

salinity levels of up to 75 mM. Helal and Mengel (1979) found a significant reduction in the contents of K^+, Mg^{2+} and Ca^{2+} in both roots and shoots of young barley seedlings exposed to salinity levels of up to 80 mM. Finck (1976) found wheat plants grown on saline soils high in Na^+ content to be deficient in K^+ and Ca^{2+}.

Helal , Koch and Mengel (1975) reported a decrease in growth and uptake of ^{15}N labelled nitrogen in salinity -stressed young barley plants. In their study it was also found that though salinity inhibited the uptake of nitrogen, it promoted its incorporation into protein. Uptake of nitrate in barley seedlings was also reported to be highly inhibited by salinity by Aslam, Huffaker and Rains (1984). Huffaker and Rains (1986) identified the uptake of nitrate and ammonium as a key limiting process of growth of barley seedlings in a saline environment. They found that high levels of salt inhibited the uptake of nitrate and ammonium by damaging both nitrate and ammonium transporters. Bottacin et al. (1985) when working with NaCl-resistant and NaCl-susceptible millet genotypes (*Pennisetum americanum*) , found the uptake of both nitrate and ammonium to be inhibited in a salt-sensitive variety whereas in a salt-resistant variety only nitrate uptake was inhibited. A study carried out by Dean-Drummond et al.(1982) disclosed that nitrate influx into barley was competitively reduced by external Cl^- ions. This study as well as others (Cram, 1973; Smith, 1973) supported the notion that since Cl^- and NO_3^- have the same charges they usually compete for the same transport system from the growth medium into the roots.

2.1.2 **Primary salt injury**

2.1.2.1 Specific ion stress or primary direct salt injury.

In contrast to secondary injury due to osmotic dehydration or nutrient deficiency, direct primary direct salt injury must involve specific toxic effects of salt either directly on the external plasma membrane or after penetrating through the membrane into the protoplast (Levitt 1980). There are many examples of glycophytes, e.g. barley (Greenway 1963) soya bean (Abel, 1969), and halophytes, e.g. *Agropyron elongatum* (Greenway and Rogers, 1963), in which tolerance to NaCl has been correlated with the ability to exclude Cl^- and/or Na^+ ions from the shoots. Various mechanism are involved in the exclusion of these ions from the shoots, including active Na^+ efflux from roots (Jeschke, 1977) and absorption by specialized

xylem parenchyma cells (Yeo et al., 1977). Failure of most plant species (more especially glycophytes) to avoid accumulation of these ions in their plant tissues (mostly leaves), can result in severe injury or death. High Cl^- concentrations in expanded leaves of certain species were associated with chlorosis and death, e.g.in avocado (Bingham et al,1968) and in grapevines (Bernstein et al, 1969). Raspberries were also found to accumulate Cl^- ions more rapidly than boysenberry and blackberry and as a result they were killed or severely injured more rapidly than others (Ehlig, 1964). These observations strongly suggest that specific ion toxicity in the shoot is a crucial factor in salt stress. Paradoxically, when the ion relations of some halophytes and glycophytes are compared in a wider context, many halophytes, e.g *Atriplex vericaria* (Black, 1960) and *Scirpus maritimus* (Mercado et al., 1971) are found to accumulate Na^+ and Cl^- ions in order to adjust osmotically.

The method that has been commonly used to distinguish between secondary osmotic and primary salt injury is that of comparing the effects of isotonic salt solutions with those of organic substances. In this method it is assumed that if the salt injury is simply osmotic in nature, then all solutes should produce the same injury at the same osmotic potential. In trying to separate the osmotic from NaCl-specific effects on plant growth Termaat an Munns (1986) grew barley, wheat and white clover in NaCl and concentrated macronutrient solutions of matching osmotic pressures. The results of their study showed that in all three species, NaCl-grown plants were less than half the size of plants grown in concentrated macronutrients, which in turn were smaller than controls (plants grown in normal strength nutrient solution). From this study it was concluded that if the effects of concentrated macronutrients are regarded to be osmotic, then the additional effects of NaCl are due to toxic effects of Na^+ and Cl^- ions.

At high external solution osmotic pressure, growth of beans was substantially better in polyethylene glycol (PEG) than in isosmotic NaCl-containing solutions. Poorer growth in NaCl than PEG was regarded as convincing evidence for ion excess because PEG may also be transported to the shoot and exert its own adverse effects (Lagerwerff et al., 1961).

Growth or yield of some plant species e.g. avocado (Downton, 1978) was found to be reduced at such low concentrations of NaCl in the external solution (5 mM) that the adverse effects of low osmotic potential are implausible. This reduction of growth under these

conditions is also regarded as direct evidence for ion excess.

Some studies have indicated that the osmotic effect on the growth of plants can be reversed (Gates, 1955). Therefore, if it is assumed that the osmotic effect is reversible, the specific ion toxicity of NaCl can be measured by the lack of recovery of growth after removal of salt from the growth medium. This is supported by the study of Greenway (1962) in which, after removing NaCl from the growth medium , he could not detect any recovery in the growth rates of three varieties of barley (*Hordeum vulgaris*) seedlings. The conclusion from this study was that excessive ion accumulation was responsible for the reduced growth on saline substrate.

2.1.2.2 Primary indirect salt injury

2.1.2.2.1 *Inhibition of growth.*

According to Gale (1975), even if the salt-stressed cell eliminates the osmotic decrease in turgor and therefore in cell growth by the process of osmoregulation, there may still be a significant decrease in growth due to salt stress for the following reason:-
The plant must maintain a state of inequilibrium with its environment in order to survive. If growing in normal soil containing the usual low concentrations of nutrient ions, it must increase these concentrations and the ion balance in its protoplasm to a level suitable for the normal functioning of the cell. If growing in saline soil, it must also concentrate and balance these ions, but over and above this increase it must decrease the concentration of the Na^+ and Cl^- ions in its protoplasm below that in the soil, in order to maintain ionic concentrations and balances that support normal functioning of the cell. This maintenance of lower concentrations of salt ions in plant's protoplasm than in the surrounding soil requires the expenditure of energy that would otherwise be available for growth processes. Growth must, therefore, be decreased. Even though growth inhibition by salt stress has been confirmed experimentally, the contribution of the above mentioned energy loss still has to be established.

2.1.2.2.2 *Metabolic disturbances.*

Salt-induced growth inhibition is accompanied by a disturbance of metabolic reactions such as photosynthesis and protein synthesis (Levitt 1980).

Studies carried out on a variety of plants have shown that photosynthetic rates of some plants are affected by salinity whereas in others the photosynthetic rates do not seem to be affected. Rawson (1986), for example, reported a salinity-induced decrease in photosynthetic rates in wheat and barley exposed to salinity levels of up to 150 mM , while the water use efficiency was marginally affected. Helal and Mengel (1981) reported a decrease in carbon dioxide assimilation in salinity-stressed broad beans. They also found the detrimental effects of salinity on carbon dioxide assimilation to be intensified by high light intensities. Seemann and Sharkey (1986) demonstrated that in *Phaseolus vulgaris*, the rate of photosynthesis was reduced independently of stomatal closure by salinity. High levels of carbon dioxide concentrations in the atmosphere were found to minimize the adverse effects of salinity on the growth of *Zea mays* and *Xanthium strumarium* (Schwarz and Gale, 1984). These authors argued that since salinity can affect the stomatal conductance of the plants, a reduction in stomatal aperture will interfere with diffusion of carbon dioxide into the leaf, which in turn can reduce photosynthesis. If there is a high concentration of carbon dioxide in the atmosphere (more than ambient [CO_2]), more carbon dioxide can diffuse into the leaf in spite of low stomatal conductances and thus ameliorate the adverse effect of salinity.

Passera and Albuzo (1978) reported a decrease in photosynthetic rate of two wheat species (*Tritium aestivum* and *T. durum*) exposed to salinity levels of up to 50 mM. In their study the process of photorespiration was found to be stimulated by salinity. Saline-stressed plants also showed a low ratio of RuBP carboxylase to PEP carboxylase activity, showing that PEP is more tolerant of salinity than RuBP. Downton (1977) also reported a decrease of photosynthetic rate in salinity-stressed grapevines with an accumulation of intermediates of the glycolytic pathway. Ziska et al. (1990) observed a decline in CO_2 assimilation capacity in 19 year old orchards (*Prunus salicina*) exposed to salinity levels of up to 28 mM under field conditions. This decline in CO_2 assimilation capacity was found to be related to increasing leaf chloride content. Plaut et al. (1990) reported a decrease in carbon dioxide assimilation in cowpea (*Vigna anguculata*) exposed to salinity levels of up to 173 mM. This

decrease in CO_2 assimilation was attributed partly to specific sodium ion effect and partly to stomatal inhibitions. Yeo, Carpon and Flowers (1985) found a 50% decrease in photosynthetic rates of rice seedlings exposed to salinity levels of up to 50 mM. This decrease in photosynthetic rate was found to be linearly related to leaf sodium concentration. Longstreth, Bolanos and Smith (1984) found that when grown over the range of 0 to 400 mM NaCl, net carbon dioxide uptake of alligator weed (*Alternanthera philoxeroides*) was reduced by 51 %.

Lewis et al. (1989) observed no effect on the rate of photosynthesis in maize plants that were exposed to salinity levels of up to 80 mM. Terry and Waldron (1986) could not detect any salinity effect on the rate of photosynthesis in sugarbeet plants exposed to salinity levels of up to 250 mM. Robinson, Downton and Millhouse (1983) concluded that for spinach salt stress (200 mM) does not result in any major decrease in the photosynthetic potential of the leaf. In trying to find the cause of growth reduction in saline-stressed barley plants, Munns et al. (1982) found saline-stressed plants to have high concentrations of soluble carbohydrates in the elongating tissues of the growing leaf, while starch concentration was not affected. From their study it was found that even though the cause of growth reduction was located in the growing tissue, photosynthesis was unaffected and not responsible for the growth reduction.

Synthesis of proteins is one of the metabolic processes that has been shown to be affected by salinity both positively and negatively. For example, in studying protein synthesis in leaf discs of *Nicotiana rustica*, Ben-Zioni et al. (1967) found that salt stress reduced the uptake of L-Leucine and its incorporation into protein. Kahane and Poljakoff-Mayber (1968) when working with pea roots made the same observation. Lubin (1963) found in protein synthesizing cell free systems obtained from *Escherichia coli* that an addition of NaCl at a level of 175 mM considerably reduced the rate of incorporation of ^{14}C-phenylalanine into protein. Helal and Mengel (1979) reported that growth and incorporation of labelled N into protein were impaired in young barley plants exposed to salinity levels of up to 80 mM. This experiment was, however, contradictory to the previous study of Helal , Kock and Mengel (1975) in which salinization (120 mM) was reported to impair uptake of labelled N, but improved incorporation of this labelled N into the protein fraction. Weimberg (1975) reported that soluble protein concentration was not affected in the leaves of highly salinized

(100 mM) pea seedlings. Langdale et al. (1973) reported that stargrass (*Cynodon plectostachyus*) showed reduced growth rates, but enhanced protein content, when exposed to salinity. Cusido et al. (1987) found the weight and longitudinal growth of *Nicotiana rustica* grown under salinity conditions (100 mM) to be lower than the controls, while their protein content was higher.

The above mentioned studies provided contradictory results on the effect of salinity in protein synthesis. The fact that the metabolic response to salinity is probably dependent on some other factors like growth rate, age of the plant, salt-sensitivity of the species used, availability of some nutrients (especially K^+, Ca^{2+} and N), growth conditions and the level of salinity applied has been suggested as an explanation for this contradiction (Helal and Mengel, 1979).

2.2 REQUIREMENT OF SODIUM FOR GROWTH

Sodium was first shown to be essential for growth in blue-green algae (*Anabaena cylindrica*) by Allen and Arnon (1955). Not only was sodium found to be essential for growth in blue-green algae, but several studies have disclosed that sodium is also essential for growth in higher plants. Brownell and Crossland (1972) have shown six species of plants having a C_4-photosynthetic pathway to respond to the additions of salt (0.1 meq/l NaCl) into the culture solutions containing 0.08 meq/l NaCl. Chlorosis and necrosis were observed in leaves of plants that did not receive sodium. Lehr (1949) found that when spinach plants were dressed with fertilizers in the form of $NaNO_3$ and $Ca(NO_3)_2$, $NaNO_3$-fed plants were significantly bigger than $Ca(NO_3)_2$-fed plants. In 1942 when working with beet, Lehr found a positive correlation between Na^+ ion concentrations and foliage and root.s biomass. Contrary to these results, the concentration of calcium was negatively correlated with the production of foliage and root biomass. Woolley (1957) found a 12% increase in the dry weight of tomato plants with addition of sodium at the rate of 1 mM of NaCl per litre of culture solution. Brownell (1965) found that angiosperm plants (*Atriplex vesicaria*) receiving 0.02 meq/l Na_2SO_4 made favourable growth and when harvested had approximately 10 times the dry weight of plants which had not received sodium.

The role played by sodium in promoting plant growth is not fully understood. Williams (1960) suggested that Na^+ and K^+ would be required in rather large quantities to maintain

the cation-anion balance within the plant. Therefore when the supply of K^+ ions is inadequate, Na^+ ions can substitute for K^+ ions since they are both univalent cations. Another possibility is that increase in growth with additions of sodium salts is the direct effect of Na^+ ions, which are performing some specific function within the plant. Lehr (1942) found that not only were Na^+ ions responsible for replacing K^+ ions, but were serving as stimulants to foliage production of beet. In this study high foliage production was found to be associated with high Na^+ ion concentrations, irrespective of K^+ ion concentrations. Williams (1960) found spinach to absorb more K^+ ions if equal amounts of Na^+ and K^+ ions were available, but Na^+ ions were found to reduce the requirement for K^+ ions and also improved yields even when potassium ions were not limiting. These studies suggested that apart from reducing potassium requirements for plant growth, Na^+ might be having an independent role as a nutrient element.

2.3 THE ROLE OF CALCIUM IN AMELIORATING SALINITY TOXICITY

Calcium is considered to maintain the integrity of plant cell membranes and hence to prevent the free diffusion of potentially toxic ions (e.g. sodium) prevalent in a saline environment. A so-called antagonism between sodium and calcium has been noted as early as 1902 when Kearney and Cameron (1902) reported that the addition of calcium would neutralize the harmful effects of sodium on various plants. Ratner (1935) suggested that the toleration by soil-grown plants of high levels of sodium was related to the availability of calcium. At high concentrations of sodium, crops failed to grow because of a breakdown in the calcium regime of the soil, resulting in an insufficiency of calcium available as plant nutrient. Thorne (1945) and Bower and Turk (1946) performed experiments similar to those of Ratner and reached the same conclusions.

Of the more recent work using water culture, Hyder and Greenway (1965) showed that the dry weight of barley and subterranean clover increased as the ratio of calcium to sodium in the growth medium was increased. Elzam and Epstein (1969) found a highly positive correlation between growth and calcium levels in *Agropyron* species exposed to salinity. La Haye and Epstein (1969) showed that *Phaseolus vulgaris* grew well in nutrient solutions containing 50 mM sodium with 1 mM calcium also present. A later study (La Haye and Epstein, 1971) showed that calcium restricts the entry of sodium into the plant and also

transport of the ion into the shoot. Most recently, a study undertaken by Cramer, Epstein and Läuchli (1990) also disclosed the beneficial effect of high concentration of Ca^{2+} (10 mM) on the growth of both NaCl-stressed (125 mM NaCl) and KCl-stressed (125 mM KCl) barley plants.

Cramer at al. (1988) reported inhibition of root elongation in maize plants exposed to salinity levels of up to 75 mM. However addition of supplemental calcium was found to increase the root elongation rate. Considerable interest has been developed on the influence of calcium ions on selective absorption and transport of nutrient ions in plants. Using barley roots, Epstein (1961) was able to demonstrate that calcium ions are essential for the ability of the membrane to selectively absorb K^+ and Na^+ ions. He found that in the absence of calcium Na^+ interfered with K^+ absorption and vice versa. With addition of supplemental Ca^{2+} in the growth medium, absorption of K^+ was promoted whereas Na^+ absorption was inhibited, leading to an increase in K^+/Na^+ absorption ratio. The same results were reported by Jacobson et al. (1961). Kent and Lauchli (1985) found that addition of supplemental Ca (10 mM) to the saline medium (200 mM) offset the reduction of cotton seedling growth caused by NaCl , by maintaining K^+/Na^+ selectivity and adequate Ca status in the root. Recently Shah, Wainwright and Merrett (1990) found calcium to decrease the Na^+/K^+ ratio in callus of salt-stressed (150 mM) cultivars of *Medicago sativa*. This decrease in Na^+/K^+ ratio also resulted in an increase in growth. Nakamura, Tanaka, Ohta and Sakata (1990) also found the addition of Ca^{2+} to the external medium to alleviate the inhibition of root elongation and to maintain a high intracellular concentration of K^+ in the elongating region of the roots of salt-stressed mung bean plants.

Calcium has also been shown to improve the uptake of nitrogen (mainly in the form of nitrate) as well as its assimilation under saline conditions. Ward et al. (1986) reported that calcium increased the activity of NO_3-transporter under saline conditions. Increasing the calcium concentration in saline nutrient solutions resulted in an increase in NO_3^- assimilation and seedling growth in barley. Huffaker and Rains (1986) also found that addition of supplemental calcium at low concentrations was able to protect nitrate and ammonium transporters against salt injury. By protecting the nitrogen transporters, calcium was able to enhance nitrogen uptake as well as its assimilation in seedlings of salt-stressed barley.

The ability of calcium to enhance the tolerance of *P. vulgaris* is, however, a function of temperature. Ayoub (1974) showed that in cool seasons (mean maximum and minimum temperatures 32 and 16 ^{0}C respectively) calcium caused a competitive inhibition of sodium uptake and translocation. In warm seasons (mean maximum and minimum temperatures 42 and 27 ^{0}C respectively) calcium had no beneficial effects and indeed high rates of calcium applications resulted in a higher death rate.

2.4 THE ROLE OF POTASSIUM IN AMELIORATING SALINITY TOXICITY

2.4.1 Interaction between sodium and potassium.

The antagonism between sodium and potassium is also well documented. At low concentrations of K^+, Na^+ ions have been reported to decrease uptake of K^+ (Cramer et al, 1987; Hajibagheri, 1987; Silberbush and Ben-Asher, 1987). On the other hand, high concentrations of K^+ have also been found to reduce accumulation of Na^+ ions in some plants. Finck (1976) found that high concentrations of K^+ in the saline medium were able to keep the Na^+ content in wheat plants at low levels. Huffaker and Wallace (1960) when working with *Zea mays*, *Glycine max*, *Citrus jambhiri* and *Persea americana* found that a high concentration of K^+ in the growth medium inhibited Na^+ absorption, whereas low levels of K^+ stimulated Na^+ absorption. Results similar to these were previously reported by Huffaker and Wallace (1959) when working with soya bean and radish.

From the above studies it can be concluded that by maintaining a high K^+/Na^+ ratio in the saline media, Na^+ accumulation in saline-stressed plants can be reduced, thereby reducing toxic effects caused by sodium ions.

2.4.2 Enhancement of nitrogen uptake.

High concentrations of NaCl can inhibit nitrogen uptake as well as its assimilation (Huffaker and Rains, 1986; Helal and Mengel, 1979; Aslam et al., 1984). However Helal, Koch and Mengel (1975) found that potassium additions (5 and 10 mM KCl) to saline (60 and 120 mM NaCl) nutrient solution were able to enhance labelled nitrogen uptake and improve the growth of salinized barley plants. In their later experiment (Helal and Mengel, 1979), potassium

additions were reported to enhance ^{15}N-labelled nitrogen uptake and its incorporation into protein, reduce the accumulation of inorganic N and improve the growth of salinized barley plants.

One possible way by which potassium enhances the uptake of nitrogen from the saline medium is the role that it plays in the uptake of nitrogen as well as its transportation within the plant. This can well be explained by the potassium-nitrate circulation model (Lips et al., 1970) in which it was proposed that nitrate would migrate from the roots to the leaves via the xylem, accompanied by potassium (KNO_3). In the leaves nitrate will be reduced, accompanied by a concomitant synthesis and accumulation of malate. The malate would then migrate down to the roots via the phloem accompanied by potassium (as K^+-malate). In the roots malate would then be further reduced into bicarbonate (HCO_3^-), which would exchange with soil nitrate. Under saline conditions high Na^+ can replace K^+. Therefore nitrate might be transported from roots to the leaves via the xylem accompanied by Na^+, but sodium-malate will not migrate down to the roots. The immobilization of malate in the shoot will thus prevent the uptake of nitrate by the roots (Silberbush and Ben-Asher, 1987). It appears that for salinized plants to be able to absorb adequate nitrate from the growth medium, high K^+/Na^+ ratio must be maintained. So, by adding supplemental potassium in the saline growth medium, high K^+/Na^+ ratios can be attained, and this can improve nitrate uptake as well as its assimilation in saline-stressed plants.

The possibility of a K^+/Na^+ interaction in the guard cells can also arise if Na^+ is translocated to the photosynthetic tissue in sufficient quantities. Since one of the most important functions of K^+ is to regulate stomatal opening (Fischer, 1968), replacement of K^+ ions by Na^+ ions in the guard cells can have a negative effect on the proper regulation of stomatal opening. As a result photosynthesis as well as the water status of saline-stressed plants can be affected.

It could then be concluded that under saline conditions potassium could play a very significant role in ameliorating the growth of salt-sensitive crop plants possibly due to its role in nitrate uptake and its competitive ability to inhibit the uptake of Na^+ ions.

CHAPTER 3

MATERIALS AND METHODS

Seedlings of *Zea mays* L. var PNR 394 were germinated and grown in vermiculite for five days after which they were transplanted into 20 litre troughs containing a well aerated Long Ashton medium (Hewitt, 1966). Prior to planting in vermiculite, seeds were soaked in water and bubbled vigorously for 24 hours to enhance the germination process.

3.1 CONDITIONS FOR GERMINATION AND GROWTH.

Seedlings were germinated and grown in a controlled environmental chamber, under conditions suitable for plants possessing the C_4-photosynthetic pathway. Day irradiance in the chamber was between 1300 and 1500 $\mu mol.m^{-2}.s^{-1}$. Light was supplied by a mixture of H.P. Sodium (400W), HQI metal halide (400W) and incandescent (150W) lamps. Day temperature was maintained at 35 ^{0}C and night temperature at 25 ^{0}C. Daytime relative humidity was 50 % and humidity during the night was 60%. The photoperiod was 14 hours. For experiments carried out at low temperature conditions, day temperature in the chamber was maintained at 25 ^{0}C and night temperature at 20 ^{0}C.

3.2 HYDROPONICAL GROWTH

Eight plants were grown in each of the 22 litre plastic containers with holes drilled in their lids to accommodate the plants (see Plate 1). Plants were grown on well-aerated Long Ashton nutrient media modified to contain nitrate or ammonium-only as the 4 mM nitrogen source (Hewitt, 1966). Nutrient composition of the used Long Ashton solutions is shown in Table 1. Aeration was carried out by a series of air stones (one stone per tank) connected to air supplying tubes. Each air stone was placed in the centre of the tank to make sure that there was an even distribution of air in each tank. In each experiment half of the troughs contained salt-stressed plants (80 mM NaCl) and the remaining half served as NaCl-free controls. For salinity-stressed plants NaCl concentration was stepped up by 40 mM each day until a final concentration of 80 mM was reached.

Nutrient solutions were renewed a week after feeding the plants for the first time and every 4th day thereafter. Because of its tendency to oxidize, additional $FeSO_4$ (0.064 mM) was added to the growth medium every third day to ensure that there was enough supply for the plants. The pH of each nutrient medium was monitored every second day and adjusted to 5.5 + 0.05. After growth in hydroponics for 12 to 16 days, plants were harvested, divided into root and shoot and their fresh weights were immediately determined. Each root and shoot was oven-dried at 80 ^{0}C for 48 hours and the dry weight of each determined afterwards.

3.3 NUTRIENT SOLUTION OSMOLALITY DETERMINATION

The osmolality of nutrient solutions used in the study was determined to ascertain the osmolality differences between NaCl-containing and non-NaCl-containing solutions. Osmolality measurements were made using a Westcor Vapour Pressure Osmometer and were based on the principle of vapour pressure decrease occurring with addition of a solute to a solvent. This vapour pressure decrease is mathematically interrelated with the other colligative properties of the solution, including osmotic pressure. The osmometer was calibrated with each new session of measurements using standard NaCl solutions purchased from the osmometer manufacturers. These were: 100 mOs kg^{-1}, 260 mOs kg^{-1} and 1000 mOs kg^{-1}. After calibration each sample solution was applied with a syringe to a 4 mm^2 disc of ashless filter paper which had been laid onto the sample holder using forceps. The sample was then inserted into the osmometer chamber. In the chamber is a sensitive thermocouple hygrometer which operates on a thermal energy balancing principle and is controlled by the electronic circuitry of the instrument. The junction is cooled via the Peltier effect to below dew point. The heat of condensation from the solution being tested raises the junction temperature asymptotically towards dew point with the result that the temperature converges towards dew point. The output meter is proportional to this temperature depression of the thermocouple junction and thus to the dew point temperature depression in the sample chamber, which will differ depending on the osmolality of the sample, i.e. to the degree of evaporation/condensation. Each sample was measured in duplicate.

3.4 GAS EXCHANGE ANALYSIS

Gas exchange by leaves of maize plants was measured by using infra-red gas analysis. This technique is based on the ability of heteroatomic molecules to absorb specific wavelengths of infra-red radiation (IR) with each specific heteroatomic molecule having a characteristic absorption spectrum. With this system air to be analysed for the concentration of a particular heteroatomic gas is passed through an analysis tube of the infra-red gas analyser (IRGA). Infra-red radiation is then shone through the analysis tube and the reduction in IR radiation is indicative of the concentration of a particular heteroatomic gas (Long and Hällgren, 1985). To measure photosynthetic CO_2 exchange a leaf is enclosed in a leaf chamber (which is linked to the IRGA) through which air is passed. The airstreams coming in and out of the leaf chamber are then analysed by an IRGA for the comparison of CO_2 concentrations. The only heteroatomic gas normally present in air with an absorption spectrum overlapping that of CO_2 is water vapour (both molecules absorb IR in the 2.7 μm region). Since water vapour is usually present in air at much higher concentrations than CO_2 this difference does present a significant problem. This is overcome by drying the air that is to be examined or by filtering out all radiation at the wavelengths where absorption by the two gases coincides (Long and Hällgren, 1985).

In this study photosynthetic rate and transpiration rate were determined with the use of a Parkinson leaf chamber linked to an ADC LCA2 infra-red gas analyser (Analytical Development Company Ltd., Hoddesdon, England). Determinations were made on 5 separate plants belonging to each treatment, and in each plant the youngest fully matured leaf was considered. Gas exchange determinations were made at an irradiance of between 1000 and 1500 μmol m^{-2} s^{-1} and a temperature of 35 ^{0}C (for high temperature-grown plants) or 25^{0}C (for low temperature grown plants). Ambient air was supplied at a constant rate by an air supply unit with a variable area flow meter (ASU, Analytical Development Company Ltd., Hoddesdon, England). The ASU was fitted with drying columns to supply dry air to the chamber. Drierite was used as a drying agent rather than the commonly used silica gel which is able to desorb or adsorb CO_2. Ambient air was used after passing through a 25 litre buffering plastic tank in order to allow equilibration of the temperature of the outside air with that of the environmental chamber to take place and also to buffer changes in CO_2 concentrations. The flow rate of ambient air was 500 ml min^{-1}.

Photosynthetic rate was calculated using the difference in CO_2 concentration between air entering and air leaving the leaf chamber. In determining CO_2 concentrations, corrections were applied for the water vapour sensitivity of the IRGA. Since the LCA-2 did not incorporate an optical filter to remove the response to water vapour, a further correction had to be applied for the change in CO_2 concentration with the change in chamber water vapour concentration. Detailed equations for the calculation of photosynthetic rate and transpiration rate are presented in the Appendix. Water use efficiency was calculated as A/E. Gas flux determinations were made a day before the date of harvest.

Moisture content of each plant was also calculated using the following formula:-

$$\%M = \frac{(fw-dw)}{fw} \times 100$$

where %M = percentage moisture content

fw = fresh weight

and dw = dry weight

3.5 ^{15}N EXPERIMENTATION

3.5.1 ^{15}N feeding

On the day of harvest, plants were transferred from 22 litre containers to 2 litre plastic containers (also with holes drilled in the lids to accommodate the plants). Each of these was filled with Long Ashton nutrient solution containing 49.1 A%E ^{15}N (see Chapter 3 for experimental details). These experiments were also carried out in a controlled environment chamber.

3.5.2 Harvesting and milling

All ^{15}N-fed plants were harvested 8 hours after the start of the experiment. After the plants were removed from the solutions, the roots were thoroughly washed with distilled water and thereafter separated from the shoots. After determining the fresh weight, each root and shoot was oven-dried at 80 ^{0}C for 48 hours. After determining the dry weight of each root and

shoot, the dried material was milled in a Wiley mill (Arthur Thomas Co., Philadelphia, U.S.A).

3.5.3 **Kjeldahl digestions and distillations**

A 0.1 g sample from each batch of milled material (with each sample taken in duplicate) was weighed into Kjeldahl flasks with one Mercury catalyst tablet and 3 ml sulphuric acid (nitrate-free -AR) containing 34 g salicylic acid per litre. Salicylic acid was added to reduce nitrate to ammonium. Digestions were then carried out in a preheated digestion block at 375 ^{0}C until all the samples cleared (this takes about 3 hours) and one hour after sample clearance. The resulting digest was made up to 25 ml with distilled water and 2 ml aliquots taken for distillation (with each distillation done in duplicate). The ammonia present in each aliquot was distilled over in a Büchi distillation unit after alkalization with 10 ml of 50% NaOH (w/v) containing 2.5% sodium thiosulphate. Sodium thiosulphate was added to immobilize any left over mercury thus preventing it from affecting ^{15}N results. Approximately 25 ml of the distillate was collected in an erlenmeyer flask containing 2 ml of 0.02 N HCl and the amount of nitrogen present in each distillate was determined by back titrating with 0.005 N NaOH using an automatic Schott titrator. In order to prevent loss of ammonia after titration, each sample was re-acidified by adding 3 to 4 drops of 0.1 N HCl. The distillates were then heated under an airstream in order to evaporate them down to a volume suitable for ^{15}N determination.

3.5.4 **^{15}N analysis**

3.5.4.1 Sample Preparation

After being blown down to a suitable volume (approximately 2 ml) for ^{15}N determination, the samples were prepared for ^{15}N analysis on a Jasco ^{15}N-analyser (Japan Spectroscopic Co., LTD.) following the sodium hypobromite method of Faust (1967). 0.3 ml of alkaline sodium hypobromite (hypobromite serving as an oxidant), was mixed with 0.1 ml of each sample in the reaction vessel.

The mixing procedure was done under vacuum, and the mixture produced a reaction which resulted in the release of nitrogen gas as follows:-

$2NH_3 + 3NaOBr \text{ ----------} > N_2 + 3H_2O + 3NaBr.$

The nitrogen gas produced was collected in an electrode discharge tube. The vacuum system consisted of a high vacuum pump, which created a prevacuum of 0.1 KPa, connected in series with a mercury diffusion pump giving the system a final pressure of 1.0 Pa. Two liquid nitrogen cold traps were used to reduce the water vapour pressure of the system.

After the nitrogen gas was collected in the discharge tube, it was excited with a ST 200K MKII high voltage gun (Edwards High Vacuum, England) producing a pink or purple fluorescence. Failure to produce such fluorescence indicated that there was either too much gas in the discharge tube or the concentration of N_2 gas in the discharge tube was too low. Under such circumstances the sample was reprepared using a different volume of sample.

3.5.4.2 ^{15}N analysis and expression of ^{15}N results

The cover of the sample chamber on the Jasco ^{15}N analyser was opened and a discharge tube was inserted into the electrode, with its constriction positioned between the electrodes. The cover of the sample chamber was then closed to cause the sample tube to be activated. After turning the ^{15}N analyser on, a high frequency current was applied to the sample of nitrogen gas in the discharge tube resulting in emission of characteristic spectral bandheads emitted by $^{14}N^{14}N$ and $^{15}N^{14}N$ respectively. After adjusting the gain, three characteristic spectral bandheads for each isotopic molecule were photoelectrically recorded. A typical trace showing the characteristic "fingerprint" of each peak for ^{15}N enrichments below 50% is shown in Figure 1.

The percentage enrichment was calculated by the formula:-

$$\%En = \frac{100}{2G\left(\frac{N^{28}}{N^{29}}\right)+1}$$

where % En = percentage enrichment

N^{28} , N^{29} = Peak heights of $^{14}N^{14}N$ and $^{15}N^{14}N$ respectively

and G = gain

All ^{15}N enrichment figures obtained were averages of three complete traces. A standard curve was drawn up for the Jasco analyser and was used to correct the enrichment figures (Figure 2). In order to obtain the %En in excess of the natural abundance (the atom percent excess, A%E), the natural abundance (0.3663) was subtracted from the corrected %En. The ^{15}N content of a particular sample, expressed as micrograms ^{15}N per milligram dry weight (μg ^{15}N mg^{-1} dw), was calculated by multiplying the total nitrogen content (obtained from distillation procedures) by the A%E value. This was then multiplied by the average weight of the plant part (root or shoot) represented by the sample, to produce results finally as micrograms per plant part.

3.6 POTASSIUM ION DETERMINATION

3.6.1 Harvesting and milling

After growth in hydroponics, plants were harvested, divided into root and shoot and their fresh weights were immediately determined. Each root and shoot was then oven-dried at 80 ^0C for 48 hours. After determining the dry weight of each root and shoot, the dried material was milled in a Wiley mill (Arthur Thomas Co., Philadelphia, U.S.A).

3.6.2 Sample digestions and dilutions for potassium analysis

Plant shoot and root materials were digested for 80 minutes in a binary mixture of A.R. nitric (HNO_3) and perchloric ($HClO_4$) acids in a 2:1 ratio.

3.6.2.1 Predigestion in Nitric acid

A 0.2 g sample from each batch of milled material (with each sample taken in duplicate) was weighed into Kjeldahl flasks with one Selenium catalyst tablet and 2 ml of concentrated nitric acid. Predigestions were then carried out for 10 minutes in a preheated digestion block at 250 ^{0}C. As the samples were digested, brown fumes were formed and additional nitric acid (2 ml) was added until brown fuming dissipated.

3.6.2.2 2 HNO_3:1 $HClO_4$ Digest

After predigestion in nitric acid, 2 ml of perchloric ($HCLO_4$) acid was added to the digestion mixture. Digestions were then further carried until all samples cleared (this took about 80 minutes). The resulting digest was made up to 100 ml with distilled water. 3 blanks were also prepared and digested in parallel with samples. 9 working KCl standards having the concentration range 0 - 160 mg l^{-1} were also prepared. Samples were subjected to the required dilutions (1:10) before potassium ion analysis was done by flame photometry.

3.6.3 **Flame photometry**

Concentrations of K^+ were determined on diluted aliquots of the digest solution using a Varian Techtron Model 1000 Atomic Absorption Spectrophotometer (Varian Techtron PTY Ltd, Melbourne, Victoria) in the following procedure.

Sample solutions were nebulized (converted into fine aerosol) and introduced into the air-acetylene flame where they were desolvated, vaporised and atomized in fast succession. Subsequently potassium atoms were raised to excited atoms via thermal collision with the constituents of the burned flame gases. Upon their return to a lower ground electronic state, the excited potassium atoms emitted light of a characteristic wavelength. This light entered the slit of the monochromator which has previously been adjusted to a wavelength of 766.5 nm (corresponding to the maximum emission of K^+). Intensity of the light emitted by potassium atoms depends upon the concentration of these atoms in the original sample solution. This light intensity was measured (at the wavelength of 766.5 nm) by the photodetector, after which it was amplified and sent to a readout device from which recordings were taken.

3.6.4 **Calculations and expression of results**

The equation for the regression line of the standard curve shown in Figure 3 is Y = 0.65x + 6. By substituting Y in this equation it was possible to calculate the amount of potassium ion expressed as mg K^+ in a litre of the sample solution. This value was divided by 10 to get mg per 100 ml of sample solution (because the sample digest was made up to 100 ml) and multiplied by the dilution factor. Potassium content (mg) per dry plant part weight (g) was calculated by using the following formula:-

$$mgK^+ \, g^{-1}dwt = mgK^+ \, ml^{-1} \times \frac{Sample\ volume\ (ml)}{Sample\ dry\ weight\ (g)}$$

This value was further converted into mmoles K^+ per dry plant part weight (g).

3.7 **STATISTICAL ANALYSIS**

The methods used follow Zar (1984) and were run on Statgraphics statistical software (STSC, 1989). Two- way analysis of variance (Two-way Anova) was done to test if there was any significant difference between various physiological characteristics of salt-stressed and non-salt-stressed plants. One way analysis of variance (One-way Anova) was performed to test if there were significant differences among various physiological characteristics of salt-stressed or non-salt-stressed plants grown in different calcium treatments. Multiple Range Tests were performed after each two-way or one-way Anova to establish were variation lay. Where only two treatments were compared, a Student t-test was carried out to test for differences. Detailed information on statistics is presented in the Appendix.

Plate 1. 14 days old maize plants growing in hydroponics. Plants on the left side are growing in non-saline nutrient solutions whereas those on the right side are growing in saline nutrient solutions.

Table 1. Constitution of modified Long Ashton nutrient solutions (Hewitt, 1966) supplied to maize plants grown hydroponically. In experiment 1, a 2.5 mM concentration of calcium was used instead of 4 mM. In all calcium experiments different calcium concentrations were supplied as shown in Tables 3, 4, and 5. The same was done for all potassium experiments

Salt	Concentration (mM)
Macronutrients	
$MgSO_4 . 7H_2O$	1.5
K_2SO_4	2.0
$CaCl_2 . 2H_2O$	4.00
$NaH_2PO_4 . 2H_2O$	0.67
$Na_2HPO_4 . 12H_2O$	1.5
Micronutrients	
H_3BO_3	0.1388
$MnSO_4 . 4H_2O$	0.0208
$ZnSO_4 . 7H_2O$	0.0023
$CuSO_4 . 5H_2O$	0.0033
$Na_2MoO_4 . 2H_2O$	0.00025
Iron source	
$FeSO_4 . 7H_2O$	0.0899
Nitrogen	
$NaNO_3$ or NH_4CL	4.0

Figure 1. Typical emission traces from the Jasco ^{15}N analyser for ^{15}N enrichments below 50%, showing good separation of the nitrogen molecules $^{14}N^{15}N$ and $^{14}N^{14}N$ where A and B represent the peak heights of the $^{14}N^{15}N$ and $^{14}N^{14}N$ bandheads respectively.

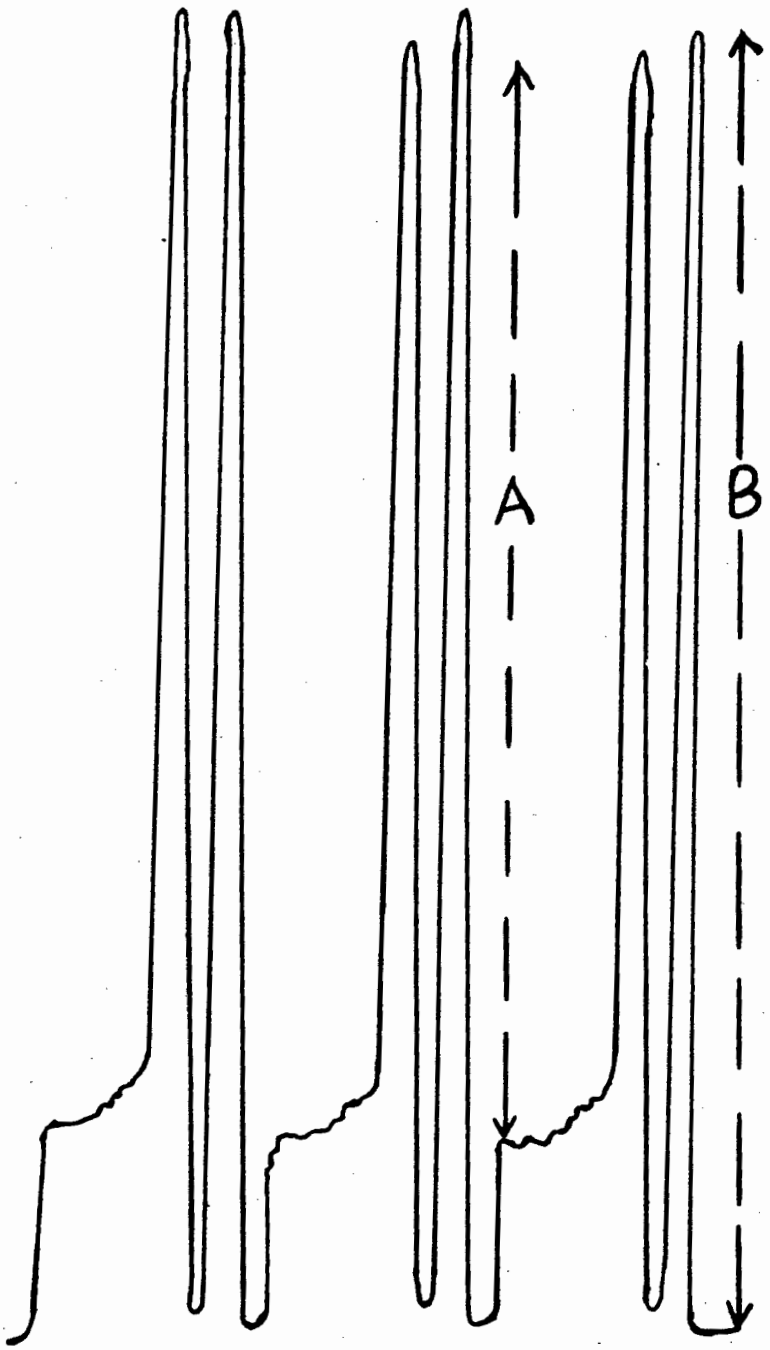


Figure 2. Standard curve for the correction of ^{15}N enrichments determined with the Jasco ^{15}N analyser.

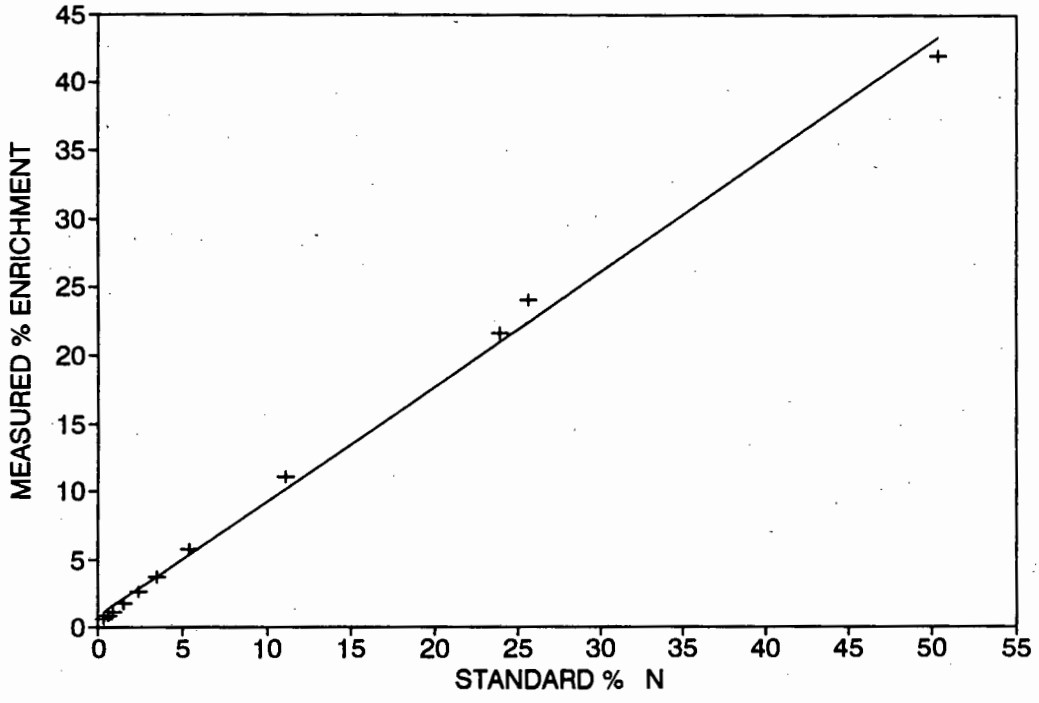
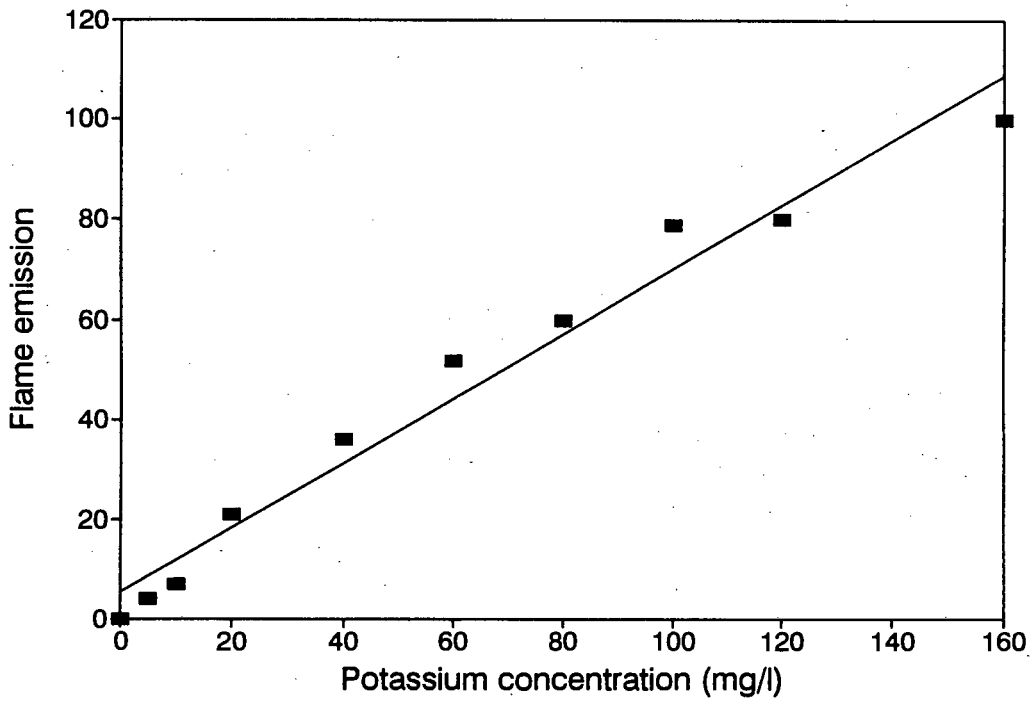


Figure 3. Standard curve for the determination of potassium concentration using a Varian Techtron Model 1000 Atomic Absorption Spectrophotometer.



CHAPTER 4

EXPERIMENTAL DETAILS

4.1. DIFFERENCES BETWEEN NITRATE- AND AMMONIUM-FED MAIZE PLANTS IN THEIR RESPONSE TO SALINITY (EXPERIMENT 1).

This experiment was carried out to establish whether nitrate-fed and ammonium-fed maize plants differ in their tolerance to salinity. This was based on the study of Lewis, Leidi and Lips (1989) who found ammonium-fed maize plants to be more sensitive to salinity than nitrate-fed plants. 16 troughs were used for the ammonium-nitrate comparison experiment. Of these 16 troughs, eight contained plants that were fed Long Ashton nutrient solution modified to contain nitrate-only as the source of 4 mM nitrogen and the other eight contained plants that were fed Long Ashton solution modified to contain 4 mM ammonium as the only source of nitrogen. For both nitrate and ammonium-receiving plants, four containers served as controls and the other four received 80 mM NaCl. All containers received 2.5 mM calcium (see Table 2). Plants were grown in Long Ashton nutrient solutions (Hewitt, 1966) for 14 days.

4.2 EFFECTS OF NaCl AND A RANGE OF CALCIUM CONCENTRATIONS (2.5 to 12 mM) ON VARIOUS PHYSIOLOGICAL CHARACTERISTICS OF NITRATE- AND AMMONIUM-FED MAIZE (EXPERIMENTS 2, 3 & 4).

4.2.1 Effects on growth and gas exchange characteristics (experiments 2 & 3)

These experiments were carried out to establish if the toxic effects of salinity on growth and gas exchange characteristics of nitrate-fed (experiment 2) and ammonium-fed (experiment 3) plants could be ameliorated by high calcium concentrations ($\pm$ 10 mM Ca^{2+}) as found in certain plant species such as cotton (Cramer et al, 1986) and barley (Cramer et al, 1990). For both experiments 2 and 3, 16 troughs were used. Half of these troughs served as controls and the remaining half (experimental troughs) received NaCl salt. Both the control and the experimental troughs were further divided into four calcium treatments (2.5, 5, 8 and 12 mM calcium) with two troughs per treatment as shown in Table 3. Plants were grown

in Long Ashton nutrient solutions (Hewitt, 1966) for 12 days.

4.2.2 Effect on ^{15}N uptake (experiment 4)

This experiment was performed in order to investigate whether nitrate uptake in maize grown under saline conditions (80 mM) could be enhanced by calcium. This was based on the findings of other researchers (Ward et al. 1980, Huffaker and Rains, 1986) who found the uptake of nitrogen in salt-stressed barley seedlings to be enhanced by calcium.

In view of the findings of other researchers and due to the high cost of ^{15}N experimentation only nitrate uptake was investigated. Due to high cost of ^{15}N, uptake of this nitrogen isotope could not be investigated in all above mentioned calcium treatments. Four 2 litre plastic containers with two plants per container were used for this experiment. Half of these containers served as controls and the remainder received 80 mM NaCl. Both the control and the experimental containers were further divided into two different calcium treatments (2.5 and 8 mM). Plants were grown in ^{15}N-labelled nitrate for 8 hours.

4.3 THE EFFECT OF A COMBINATION OF DIFFERENT TEMPERATURES (35 ^{0}C AND 25 ^{0}C) AND A RANGE OF CALCIUM CONCENTRATIONS (1 TO 8 mM) ON THE RESPONSE OF NITRATE-FED MAIZE TO SALINITY (EXPERIMENTS 5 & 6).

After not finding any ameliorative effect of the above mentioned range of calcium concentrations (2.5 to 12 mM), experiments 5 & 6 were performed to establish if the effect of salinity toxicity on the various physiological characteristics of maize could be ameliorated by this range of calcium concentrations (1 to 8 mM). Ameliorative effects of this range of calcium concentrations (1 to 8 mM) on various physiological characteristics of salt-stressed maize grown at 35 ^{0}C (experiment 5) and at 25 ^{0}C (experiment 6) were studied in order to establish if the role of calcium in promoting plant growth under saline conditions could be a function of temperature. This was based on the study of Ayoub (1974), who when working with a similar range of calcium concentrations, found that in cool seasons calcium caused a competitive inhibition of sodium uptake and translocation which resulted in improved growth of salt-stressed bean (*Phaseolus vulgaris*) plants whereas in warm seasons calcium did not show any ameliorative effect on the growth of these plants. Experiments 5 and 6 were

carried out in a way similar to that described for experiments 2 & 3. However in these experiments (5 & 6), instead of 2.5 , 5, 8 and 12 mM, the various calcium treatments were replaced with 1, 2.5, 5 and 8 mM Ca respectively (see Table 4). Plants were grown in Long Ashton nutrient solutions (Hewitt, 1966) for 14 days.

4.4 THE EFFECT OF A COMBINATION OF DIFFERENT TEMPERATURES (35 ^{0}C AND 25 ^{0}C) AND A RANGE OF CALCIUM CONCENTRATIONS (0.5 TO 5 mM) ON THE RESPONSE OF NITRATE-FED MAIZE TO SALINITY (EXPERIMENTS 7, 8 & 9).

4.4.1 Effects on growth and gas exchange characteristics (experiments 7 & 8)

After failing to find any ameliorative effect of the above mentioned range of calcium concentrations (1 to 8 mM), experiments 7 and 8 were carried out to find out whether salinity toxicity on growth and gas exchange characteristics of maize could be ameliorated by a range of low calcium concentrations (0.5 to 5 mM) as found in other plant species such as bean (La Haye and Epstein, 1969) and barley (Huffaker and Rains, 1986). Still based on the study of Ayoub (1974), maize plants were grown at 35 ^{0}C (experiment 7) and 25 ^{0}C (experiment 8) to establish if the ameliorative effects of calcium on growth of salt-stressed maize could be temperature dependent. 12 troughs were used for experiments 7 and 8. Half of these troughs served as controls and the remaining half received NaCl salt. Both the control and experimental troughs were further divided into three calcium treatments (0.5, 1 and 5 mM Ca^{2+}) with two troughs per treatment (see Table 5). Plants were grown in Long Ashton nutrient solutions (Hewitt, 1966) for 16 days.

4.4.2 Effect of NaCl on nitrate uptake at 25 ^{0}C (experiment 9)

When grown in nitrate-containing nutrient solutions at 25 ^{0}C, salt-stressed maize plants supplied with 0.5 mM Ca^{2+} grew significantly larger than non-salt-stressed plants ($p < 0.05$, t-test). It was hypothesized that the uptake of nitrogen into the roots and shoots of maize plants supplied with 0.5 Ca^{2+} at 25 ^{0}C was enhanced by salinity. This enhancement of nitrogen uptake by salinity was thought to be the reason for higher growth of salt-stressed plants as compared to non-salt-stressed plants. This ^{15}N uptake experiment was carried out

to test the above mentioned hypothesis. Four 2 litre plastic containers with two plants per container were used for this experiment. Two of these containers were used as controls and the remaining two received 80 mM NaCl. Plants were grown in ^{15}N-labelled nitrate for 8 hours.

4.5 EFFECT OF POTASSIUM ON THE RESPONSES OF NITRATE-FED (EXPERIMENT 10) AND AMMONIUM-FED (EXPERIMENT 11) MAIZE TO SALINITY

Helal, Koch and Mengel (1975) and Helal and Mengel (1979) found that potassium additions to saline nutrient solution were able to enhance nitrogen uptake and improve growth of salinized barley plants. Experiments 10 and 11 were performed to find out if potassium could alleviate the toxic effects of salinity on various physiological characteristics of nitrate- and ammonium-fed maize. For both experiments 10 and 11, 16 troughs were used. Half of these served as controls and the remaining half received NaCl salt. Both the experimental and control troughs were further divided into different potassium treatments (0.2, 1, 2.5 and 5 mM K^+) with two troughs per treatment (see Table 6). Plants were grown in Long Ashton nutrient solutions (Hewitt, 1966) for 16 days.

Table 2. Experimental design for experiment 1. This experiment was performed to investigate the difference between nitrate- and ammonium-fed maize plants in their tolerance to salinity.

Calcium concentration (mM)	NaCl concentration (mM)	Number of tanks	Number of plants/tank
	NITRATE-FED PLANTS		
2.5	0	4	8
	80	4	8
	AMMONIUM-FED PLANTS		
2.5	0	4	8
	80	4	8

Table 3. Experimental design for experiment 2 and 3. These experiments were performed in order to investigate whether the toxic effects of NaCl on various physiological characteristics of nitrate-fed (experiment 2) and ammonium-fed (experiment 3) maize plants could be ameliorated by this range of calcium concentrations (2.5 to 5 mM).

Calcium conc. (mM)	NaCl conc. (mM)	Number of troughs	Number of plants/trough
2.5	0	2	8
	80	2	8
5	0	2	8
	80	2	8
8	0	2	8
	80	2	8
12	0	2	8
	80	2	8

Table 4. Experimental designs for experiments 5 and 6. These experiments were performed to investigate whether salinity toxicity on various physiological characteristics of nitrate-fed plants grown at 35 °C (experiment 5) and at 25 °C (experiment 6) could be ameliorated by this range of calcium concentrations (1 to 8 mM).

Calcium conc. (mM)	NaCl conc. (mM)	Number of troughs	Number of plants/trough
1	0	2	8
	80	2	8
2.5	0	2	8
	80	2	8
5	0	2	8
	80	2	8
8	0	2	8
	80	2	8

Table 5. Experimental design for experiments 7 and 8. These experiments were performed to investigate whether salinity toxicity on the various physiological characteristics of maize plants grown at 35 °C (experiment 7) and at 25 °C (experiment 8) could be ameliorated by this range of calcium concentrations (0.5 to 5 mM).

Calcium conc. (mM)	NaCl conc. (mM)	Number of troughs	Number of plants/trough
0.5	0	2	8
	80	2	8
1	0	2	8
	80	2	8
5	0	2	8
	80	2	8

Table 6. Experimental design for experiments 10 and 11. These experiments were performed to investigate whether salinity toxicity on various physiological characteristics of nitrate-fed (experiment 10) and ammonium-fed (experiment 11) maize plants could be ameliorated by a range of potassium concentrations (0.2 to 5 mM).

Potassium conc. (mM)	NaCl conc. (mM)	Number of troughs	Number of plants/trough
0.2	0	2	8
	80	2	8
1	0	2	8
	80	2	8
2.5	0	2	8
	80	2	8
5	0	2	8
	80	2	8

CHAPTER 5

RESULTS

5.1. DIFFERENCES BETWEEN NITRATE- AND AMMONIUM-FED MAIZE PLANTS IN THEIR RESPONSE TO SALINITY.

5.1.1 Growth response

The dry weights of shoots and roots of nitrate- and ammonium-fed plants grown in non-saline (0 mM NaCl) and saline (80 mM) nutrient media are shown in Figure 4. From this figure it can be seen that there was no significant difference ($P > 0.05$, t-test) between the dry weights of shoots and roots of nitrate-fed plants and ammonium-fed plants grown in non-saline nutrient media. Under non-saline conditions the dry weight shoot:root ratio of ammonium-fed plants was significantly larger than that of nitrate-fed plants ($p < 0.05$, t-test). Under saline conditions, nitrate-fed plants grew significantly larger than ammonium-fed plants ($p < 0.05$, t-test). It was also observed that while all nitrate-fed plants were still growing under saline conditions, many of the ammonium-fed plants were showing signs of severe leaf damage and some were actually dying. The leaves of salt-stressed ammonium-fed plants changed colour from green to yellowish green indicating chlorophyll damage whereas leaf colour of nitrate-fed plants was not affected by salinity. The growth of both nitrate- and ammonium-fed plants was significantly reduced by salinity ($p < 0.05$, t-test). Salinity caused 27 % and 59% shoot growth reduction in nitrate- and ammonium-fed plants respectively. Root growth of nitrate- and ammonium-fed plants was reduced by 34 % and 60 % respectively. In both ammonium- and nitrate-fed plants the dry weight shoot:root ratios of salt-stressed plants were not significantly different from those of non-salt-stressed plants ($p > 0.05$, t-test).

5.1.2 Gaseous exchange response

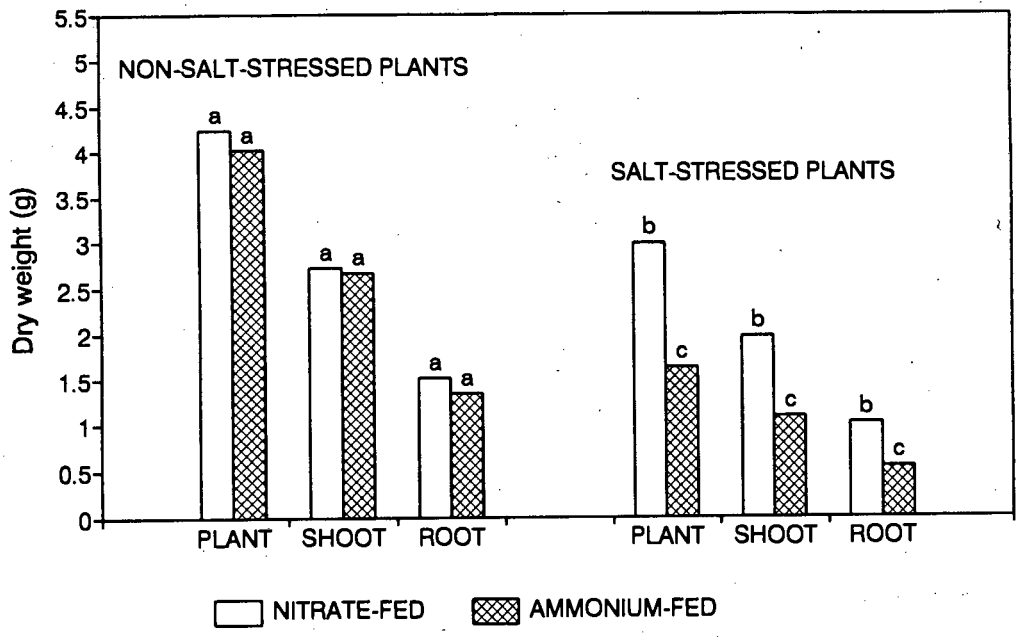
The photosynthetic rates, transpiration rates and water use efficiencies of nitrate- and ammonium-fed plants grown in saline and non-saline nutrient media are shown in Figures 5 and 6. Under non-saline conditions, photosynthetic rates, transpiration rates and water use

efficiencies of nitrate-fed plants were not significantly different from those of ammonium-fed plants ($p > 0.05$, t-test). When grown in saline nutrient media, the values of these properties in ammonium-fed plants were significantly lower than those of nitrate-fed plants ($p < 0.05$, t-test). In salt-stressed plants supplied with nitrate they were not significantly different from those of their non-salt-stressed controls ($p < 0.05$, t-test). On the other hand in salt-stressed plants supplied with ammonium they were significantly lower than in their non-salt-stressed controls ($p < 0.05$, t-test).

5.1.3 **Potassium content response**

Potassium contents of shoots and roots of nitrate- and ammonium-fed plants grown in saline (80 mM) and non-saline nutrient media are shown in Figure 7. From this figure it can be seen that when grown in saline and non-saline nutrient media, potassium contents of shoots and roots of ammonium-fed plants were significantly lower than those of nitrate-fed plants ($p < 0.05$, t-test). In both nitrate- and ammonium-fed plants, potassium contents of shoots and roots of salt-stressed plants were significantly lower than those of non-salt-stressed plants ($p < 0.05$, t-test).

Figure 4. **Dry weights of whole plants, shoots and roots and dry shoot:root ratios of salt-stressed and non-salt-stressed maize supplied either nitrate or ammonium.** Results are presented as means of 32 replicates. Similar letters indicate non-significant differences between treatments ($P > 0.05$, t-test) whereas different letters indicate significant differences ($P < 0.05$, t-test) between treatments.



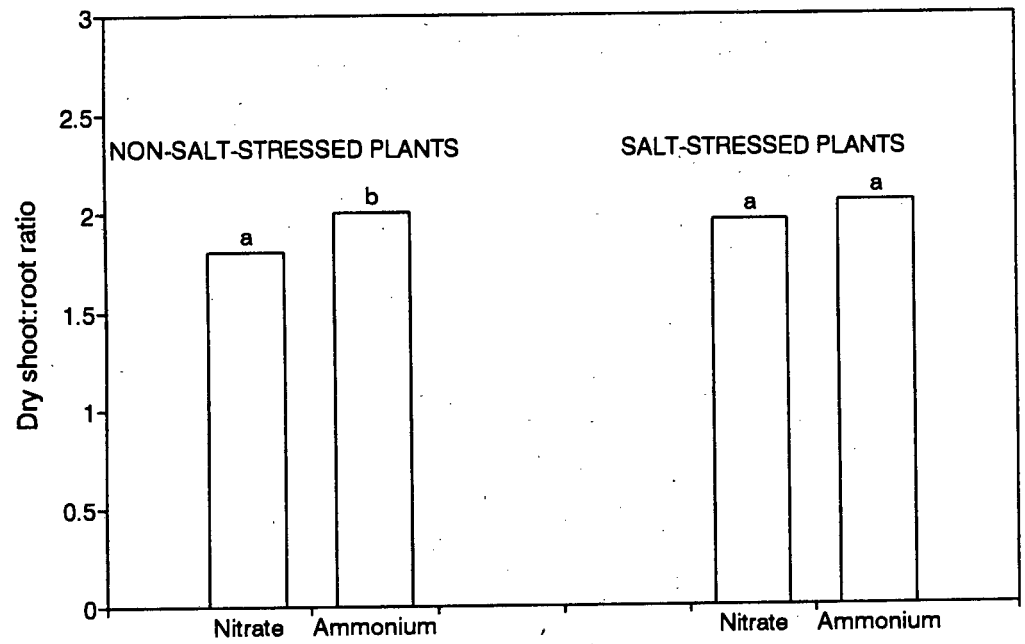


Figure 5. **Photosynthetic rates and transpiration rates of salt-stressed and non-salt-stressed maize supplied either nitrate or ammonium.** Results are presented as means of 10 replicates. Similar letters indicate non-significant differences between treatments ($P > 0.05$, t-test) whereas different letters indicate significant differences ($P < 0.05$, t-test) between treatments

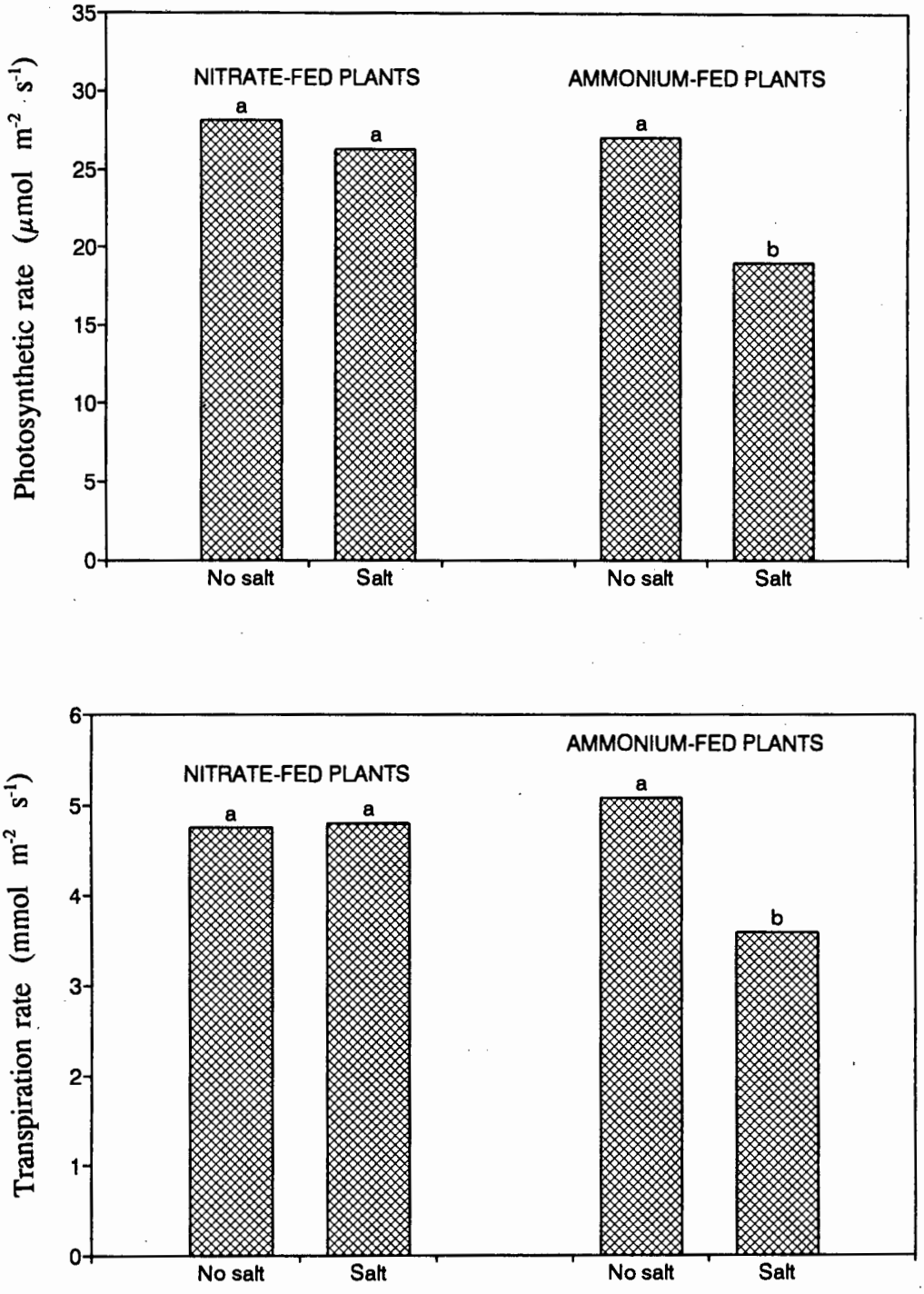


Figure 6. **Water use efficiencies of salt-stressed and non-salt-stressed maize supplied either nitrate or ammonium.** Results are presented as means of 10 replicates. Similar letters indicate non-significant differences between treatments ($P > 0.05$, t-test) whereas different letters indicate significant differences ($P < 0.05$, t-test) between treatments.

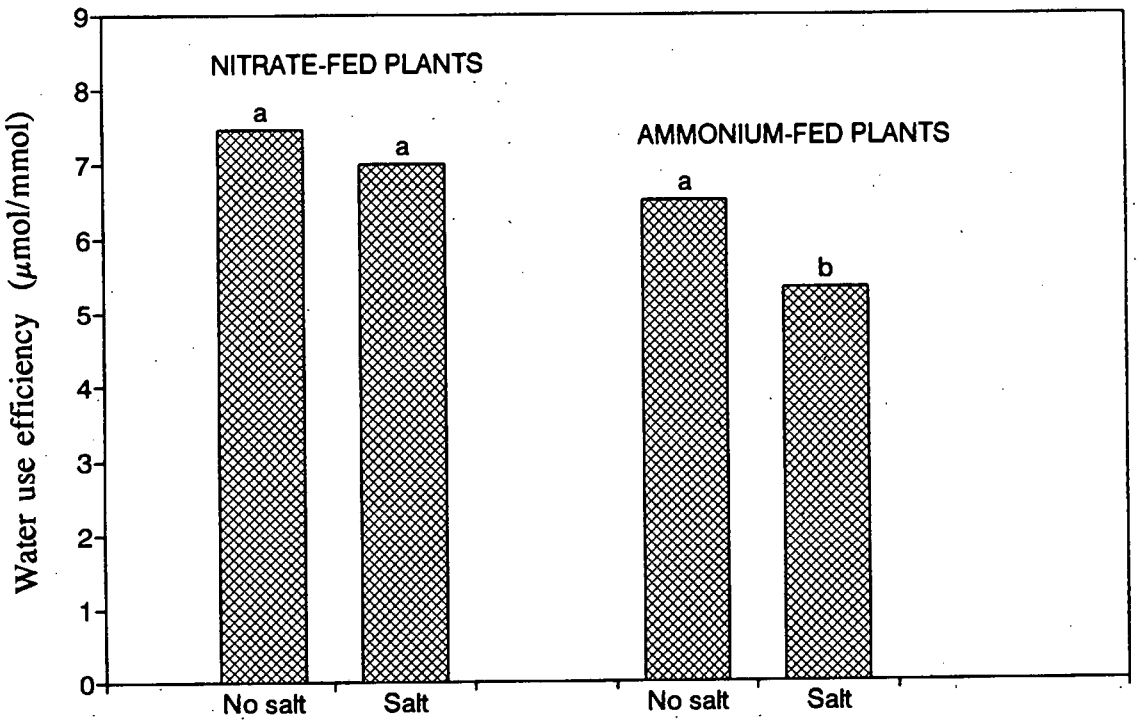
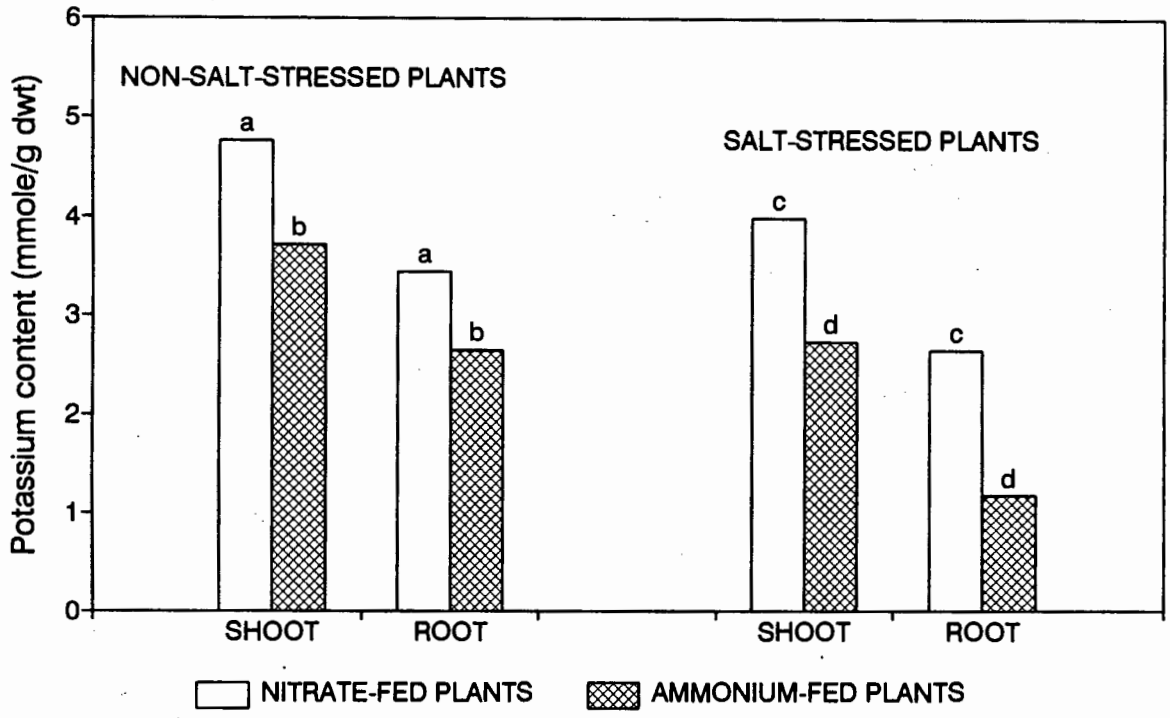


Figure 7. **Potassium contents of shoots and roots of salt-stressed and non-salt-stressed maize supplied either nitrate or ammonium.** Results are presented as means of 8 replicates. Similar letters indicate non-significant differences between treatments ($P > 0.05$, t-test) whereas different letters indicate significant differences ($P < 0.05$, t-test) between treatments.



5.2 EFFECTS OF NaCl AND A RANGE OF CALCIUM CONCENTRATIONS (2.5 to 12 mM) ON VARIOUS PHYSIOLOGICAL CHARACTERISTICS OF NITRATE- AND AMMONIUM-FED MAIZE.

5.2.1 Gas exchange response

Photosynthetic rates and transpiration rates of salt-stressed and non-salt-stressed plants grown in nitrate- and ammonium-containing nutrient media supplemented with different calcium concentrations are shown in Figures 8 and 9. Photosynthetic rates and transpiration rates of nitrate-fed maize plants were not significantly affected by salinity ($P > 0.05$, Two-way Anova) whereas those of ammonium-fed maize plants were significantly reduced by salinity ($p < 0.05$, Two-way Anova). In both nitrate- and ammonium-fed plants there was no statistically significant effect of calcium on photosynthetic rates and transpiration rates of both salt-stressed and non-salt-stressed plants ($P > 0.05$, One-way Anova).

5.2.2. Nitrate uptake response

The ^{15}N-labelled nitrogen contents of whole plants, shoots and roots of salt-stressed and non-salt-stressed plants grown in ^{15}N-labelled nitrate-containing media supplemented with different calcium concentrations are shown in Figure 10. From these figure it can be seen that the ^{15}N contents of whole plants, shoots and roots were significantly reduced by salinity ($p < 0.05$, Two-way Anova). In both salt-stressed and non-salt-stressed plants calcium did not show any significant effect on the ^{15}N contents of whole plants, shoots and roots ($p > 0.05$, t-test).

5.2.3 Moisture content response

The moisture contents of whole plants, shoots and roots of salt-stressed and non-salt-stressed maize grown in nitrate- and ammonium-containing media supplemented with different calcium concentrations are shown in Figure 11. In both nitrate- and ammonium-fed plants, the moisture contents of shoots, roots and whole plants were significantly reduced by salinity ($p < 0.05$, two-way Anova). In both ammonium- and nitrate-fed plants calcium concentrations did not show any significant effect on moisture contents of whole plants, shoots and roots of salt-stressed and non-salt-stressed plants (one-way Anova, $p > 0.05$).

5.2.4 **Growth response**

The dry weights of whole plants, shoots and roots of salt-stressed and non-salt-stressed plants grown in nitrate- and ammonium-containing media supplemented with different calcium concentrations are shown in Figure 12. In both nitrate- and ammonium-fed plants the dry weights of whole plants, shoots and roots were significantly reduced by salinity ($p < 0.05$, two-way Anova). In both nitrate- and ammonium-fed plants calcium concentrations did not have any significant effect on the dry weights of non-salt-stressed whole plants, shoots, and roots ($p > 0.05$, one-way Anova). Under non-saline conditions the growth of nitrate-fed plants was not significantly different from that of ammonium-fed plants ($p > 0.05$, two-way Anova) whereas under saline conditions nitrate-fed plants grew significantly larger than ammonium-fed plants ($p < 0.05$, two-way Anova). At the calcium concentration of 8 mM and below the dry weights of salt-stressed plants fed nitrate or ammonium were the same whereas 12 mM Ca^{2+} significantly reduced the dry weights of these plants ($p < 0.05$, One-way Anova).

Figure 8. **Effect of a range of calcium concentrations (2.5 to 12 mM) on the photosynthetic rates of salt-stressed and non-salt-stressed maize supplied NITRATE or AMMONIUM.** Results are presented as means of 5 replicates for each calcium treatment in both salt-stressed and non-salt-stressed plants. In both salt-stressed and non-salt-stressed plants similar letters show non-significant differences among different calcium treatments ($p > 0.05$, One-way Anova) whereas different letters show significant differences among different calcium treatments ($p < 0.05$, One-way Anova).

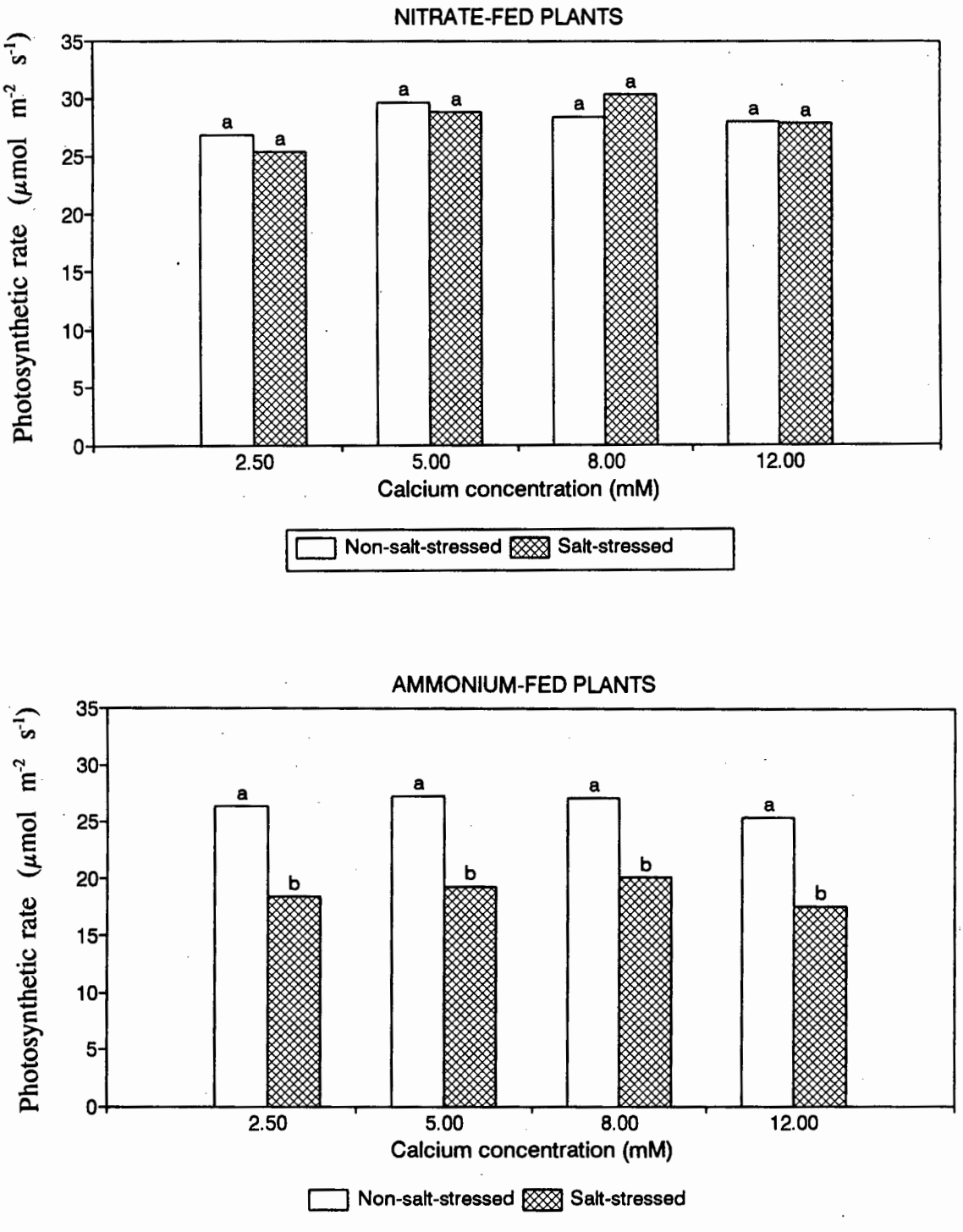
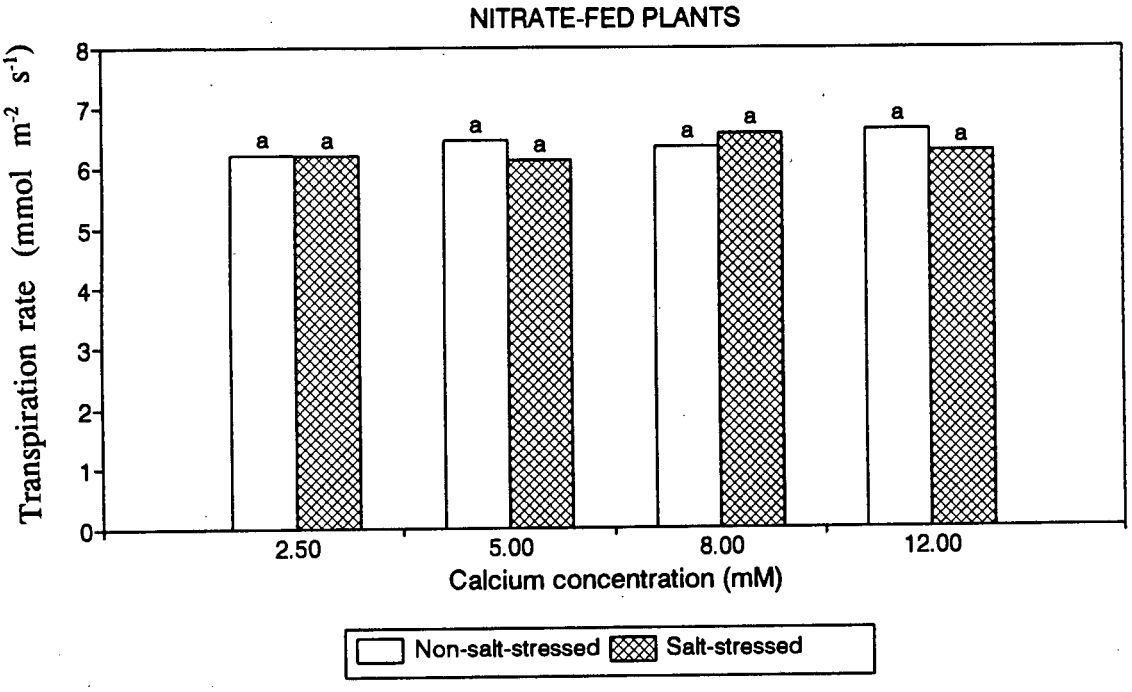


Figure 9. **Effect of a range of calcium concentrations (2.5 to 12 mM) on the transpiration rates of salt-stressed and non-salt-stressed maize supplied NITRATE or AMMONIUM.** Results are presented as means of 5 replicates for each calcium treatment in both salt-stressed and non-salt-stressed plants. In both salt-stressed and non-salt-stressed plants similar letters show non-significant differences among different calcium treatments ($p > 0.05$, One-way Anova) whereas different letters show significant differences among different calcium treatments ($p < 0.05$, One-way Anova).



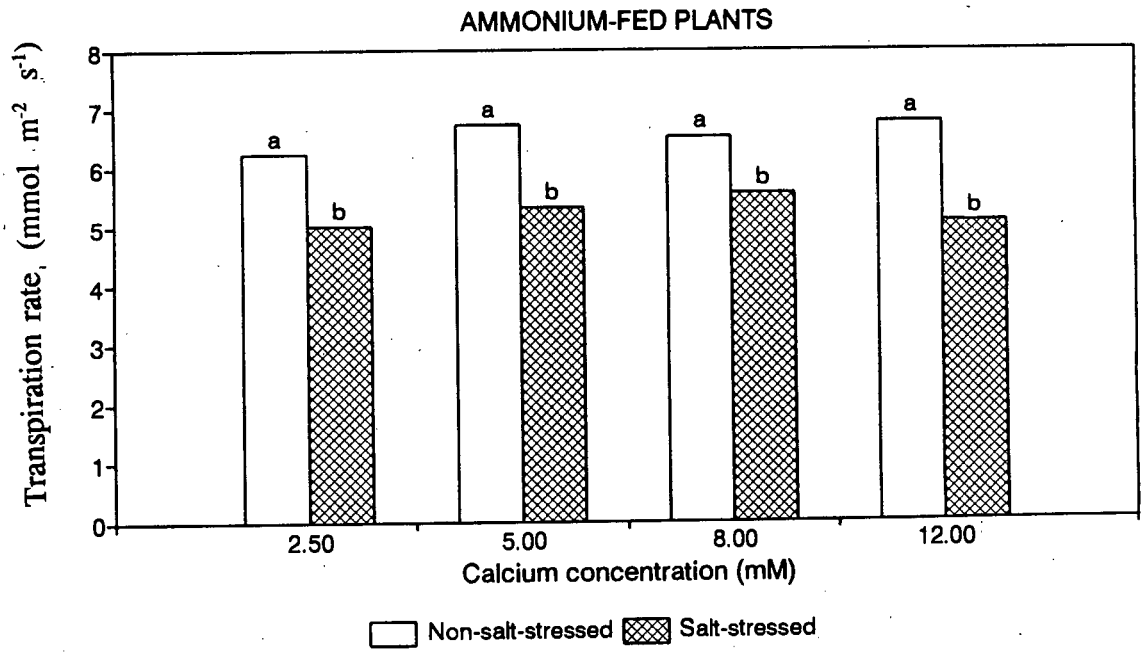


Figure 10. **Effect of calcium concentration on the ^{15}N contents of WHOLE PLANTS, SHOOTS and ROOTS of salt-stressed and non-salt-stressed maize supplied NITRATE.** In both salt-stressed and non-salt-stressed plants results are presented as means of 4 replicates for each calcium treatment. In both salt-stressed and non-salt-stressed plants similar letters show non-significant differences between the two different calcium treatments ($p > 0.05$, t-test) whereas different letters show significant differences between the two different calcium treatments ($p < 0.05$, t-test).

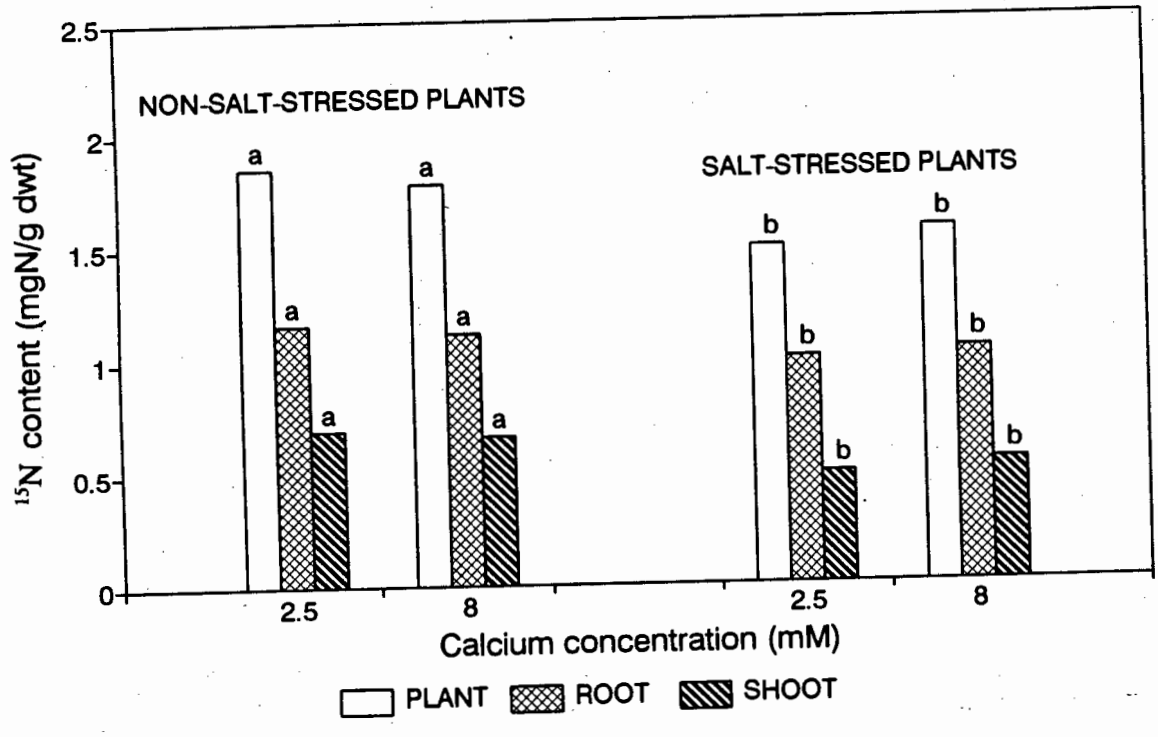
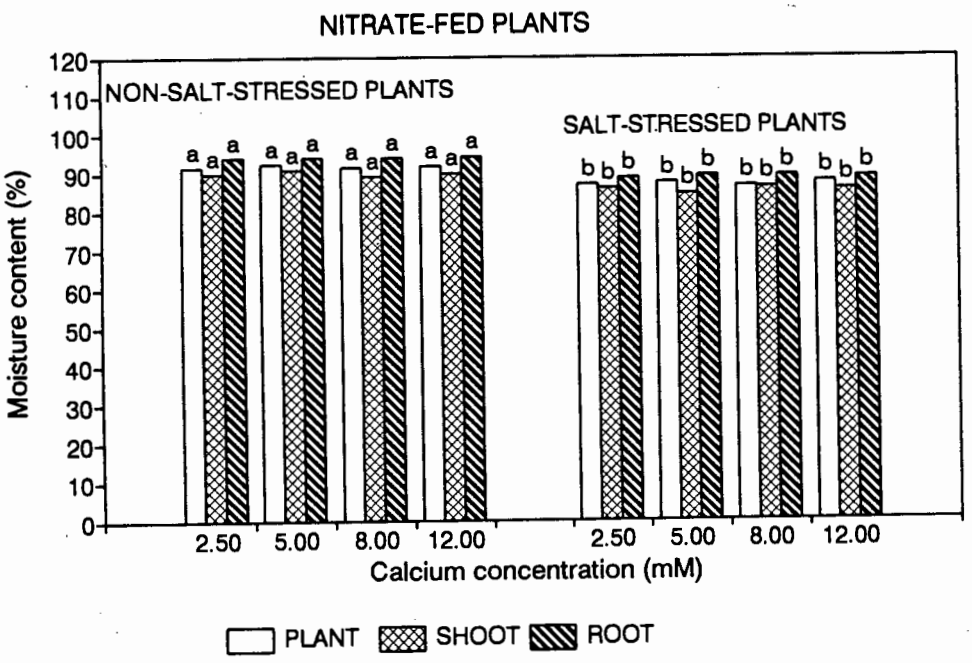


Figure 11. **Effect of a range of calcium concentrations (2.5 to 12 mM) on the moisture contents of WHOLE PLANTS, SHOOTS and ROOTS of salt-stressed and non-salt-stressed maize supplied NITRATE or AMMONIUM.** Results are presented as means of 16 replicates for each calcium treatment in both salt-stressed and non-salt-stressed plants. In both salt-stressed and non-salt-stressed plants similar letters show non-significant differences among different calcium treatments ($p > 0.05$, One-way Anova) whereas different letters show significant differences among different calcium treatments ($p < 0.05$, One-way Anova).



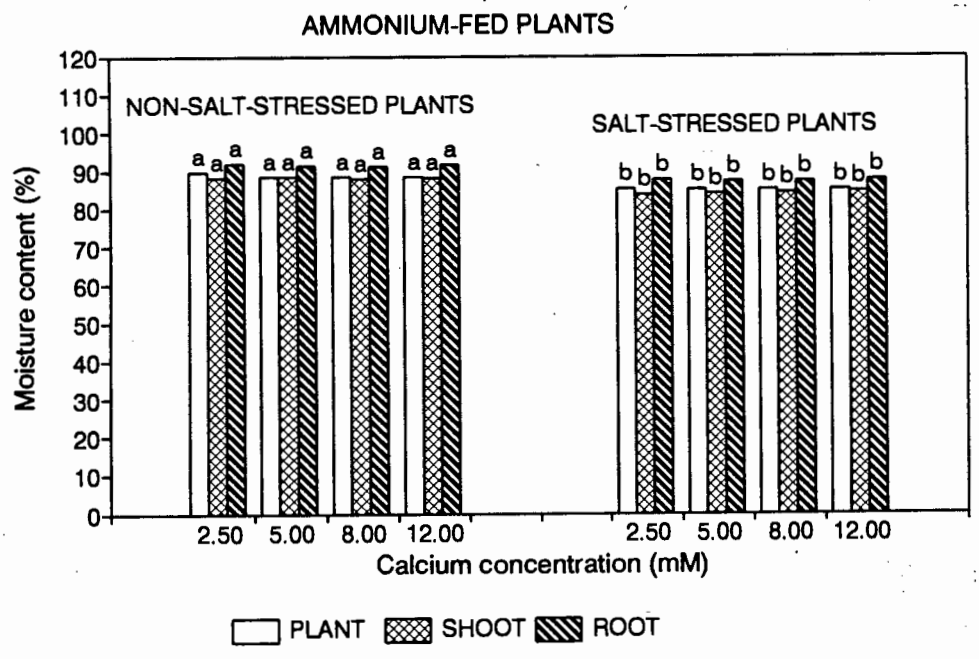
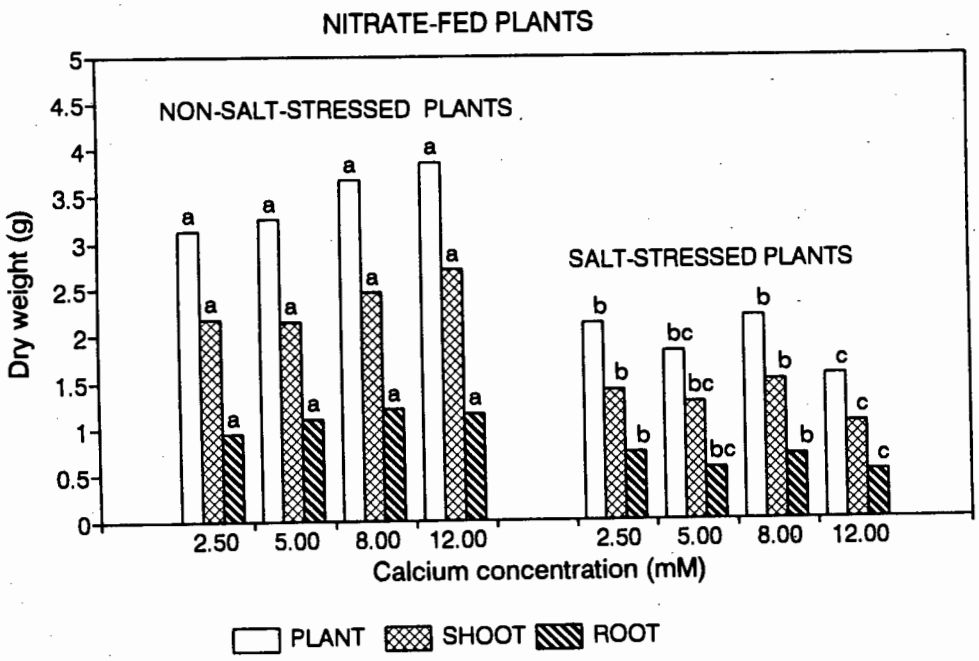
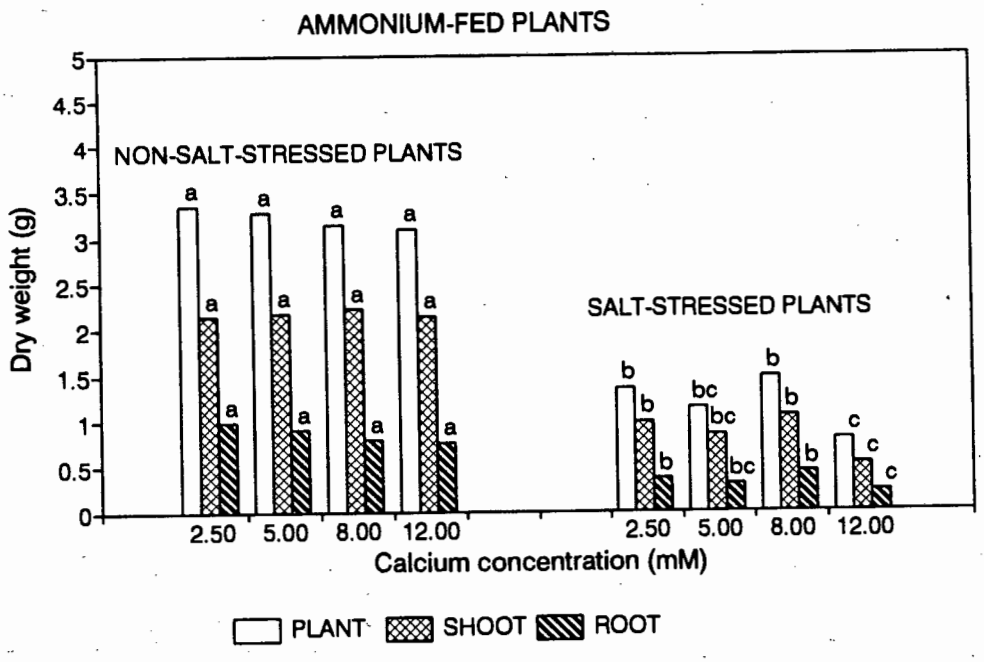


Figure 12. **Effect of a range of calcium concentrations (2.5 to 12 mM) on the dry weights of WHOLE PLANTS, SHOOTS and ROOTS of salt-stressed and non-salt-stressed maize supplied NITRATE or AMMONIUM.** Results are presented as means of 16 replicates for each calcium treatment in both salt-stressed and non-salt-stressed plants. In both salt-stressed and non-salt-stressed plants similar letters show non-significant differences among different calcium treatments ($p > 0.05$, One-way Anova) whereas different letters show significant differences among different calcium treatments ($p < 0.05$, One-way Anova).





5.3 THE EFFECT OF A COMBINATION OF DIFFERENT TEMPERATURES (35 ^{0}C AND 25 ^{0}C) AND A RANGE OF CALCIUM CONCENTRATIONS (1 TO 8 mM) ON THE RESPONSE OF NITRATE-FED MAIZE TO SALINITY.

5.3.1 Gaseous exchange response

Photosynthetic rates and transpiration rates of salt-stressed and non-salt-stressed plants grown at either 35 ^{0}C or 25 ^{0}C in nutrient media supplemented with different calcium concentrations are shown in Figures 13 and 14. From these figures it can be seen that the photosynthetic rates and transpiration rates of these plants did not show any significant response to salinity ($p > 0.05$, Two-way Anova). Increasing the calcium concentration from 1 to 8 mM did not have any significant effect on either photosynthetic rates or transpiration rates of salt-stressed and non-salt-stressed plants (One-way Anova, $p > 0.05$). For both salt-stressed and non-salt-stressed plants the photosynthetic rates and transpiration rates of maize plants grown at 35 ^{0}C were significantly higher than those of plants grown at 25 ^{0}C ($p < 0.05$, Two-way Anova).

5.3.2 Moisture content response

Moisture contents of shoot and root of nitrate-fed plants grown at both 35 ^{0}C and 25 ^{0}C are shown in Figure 15. In plants grown at both 35 ^{0}C and 25 ^{0}C the moisture contents of shoots and roots were significantly reduced by salinity ($p < 0.05$, Two-way Anova). In plants grown at both 35 ^{0}C and 25 ^{0}C increasing the calcium concentration from 1 to 8 mM did not have any significant effect on the moisture contents of shoots and roots. For both salt-stressed and non-salt-stressed plants there was no significant difference between the moisture contents of shoots, roots and whole plants grown at 35 ^{0}C and those of plants grown at 25 ^{0}C ($p > 0.05$, Two-way Anova).

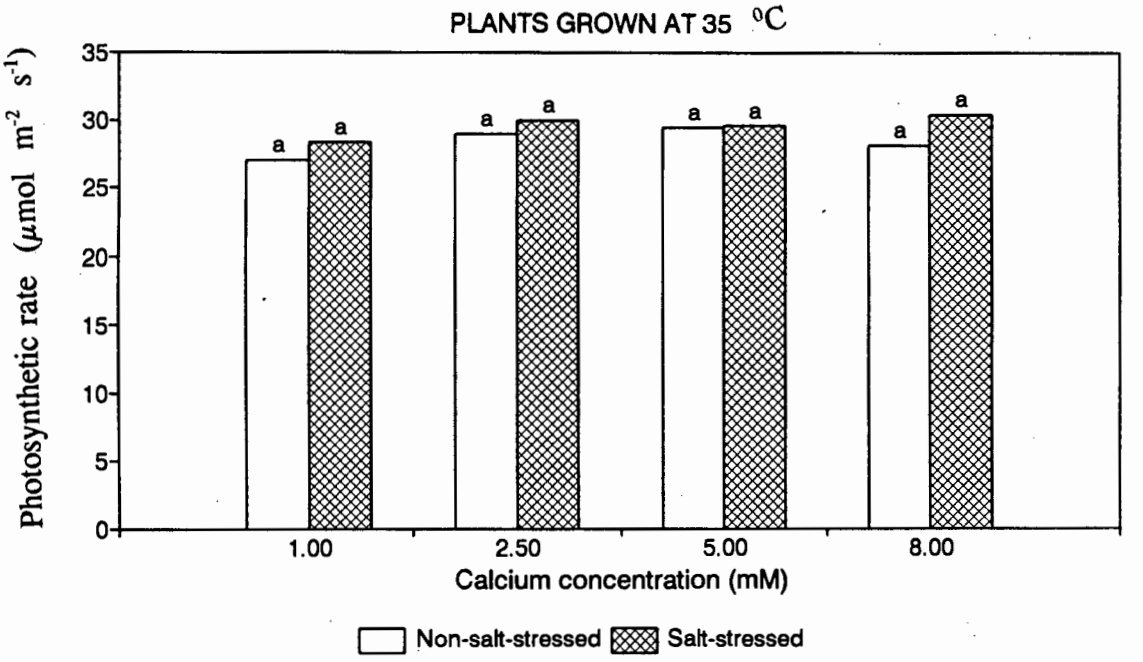
5.3.3 Growth response

Dry weights of shoots and roots of salt-stressed and non-salt-stressed maize grown at 35 ^{0}C and at 25 ^{0}C in nutrient media supplemented with different calcium concentrations are shown in Figure 16. The dry weights of shoots and roots of nitrate-fed plants grown at 35 ^{0}C were

significantly reduced by salinity ($p < 0.05$, Two-way Anova). When grown at 25 ^{0}C, the dry weights of shoots and roots of nitrate-fed plants were not significantly reduced by salinity (Two-way Anova, $p > 0.05$). When supplied with a low calcium concentration (1 mM Ca^{2+}), salt-stressed plants grew significantly larger than non-salt-stressed plants ($p < 0.05$, t-test). Increasing the calcium concentration from 1 to 8 mM resulted in a significant increase in growth of non-salt-stressed plants grown at 35 ^{0}C and at 25 ^{0}C (One-way Anova, $p < 0.05$). On the other hand increased calcium concentration did not show any beneficial effect on the growth of salt-stressed plants grown at either 35 ^{0}C or 25 ^{0}C (One-way Anova, $p > 0.05$).

Under non-saline conditions the dry weights of maize plants grown at 35 ^{0}C were significantly larger than those of plants grown at 25 ^{0}C ($p < 0.05$, two-way Anova) whereas under saline conditions the dry weights of maize plants grown at 35 ^{0}C were not significantly different from those of plants grown at 25 ^{0}C ($p > 0.05$, two-way Anova).

Figure 13. **Effect of a range of calcium concentrations (1 to 8 mM) on the photosynthetic rates of salt-stressed and non-salt-stressed maize supplied nitrate at 35 ^{0}C and at 25 ^{0}C.** Results are presented as means of 5 replicates for each calcium treatment in both salt-stressed and non-salt-stressed plants. In both salt-stressed and non-salt-stressed plants similar letters show non-significant differences among different calcium treatments ($p > 0.05$, One-way Anova) whereas different letters show significant differences among different calcium treatments ($p < 0.05$, One-way Anova).



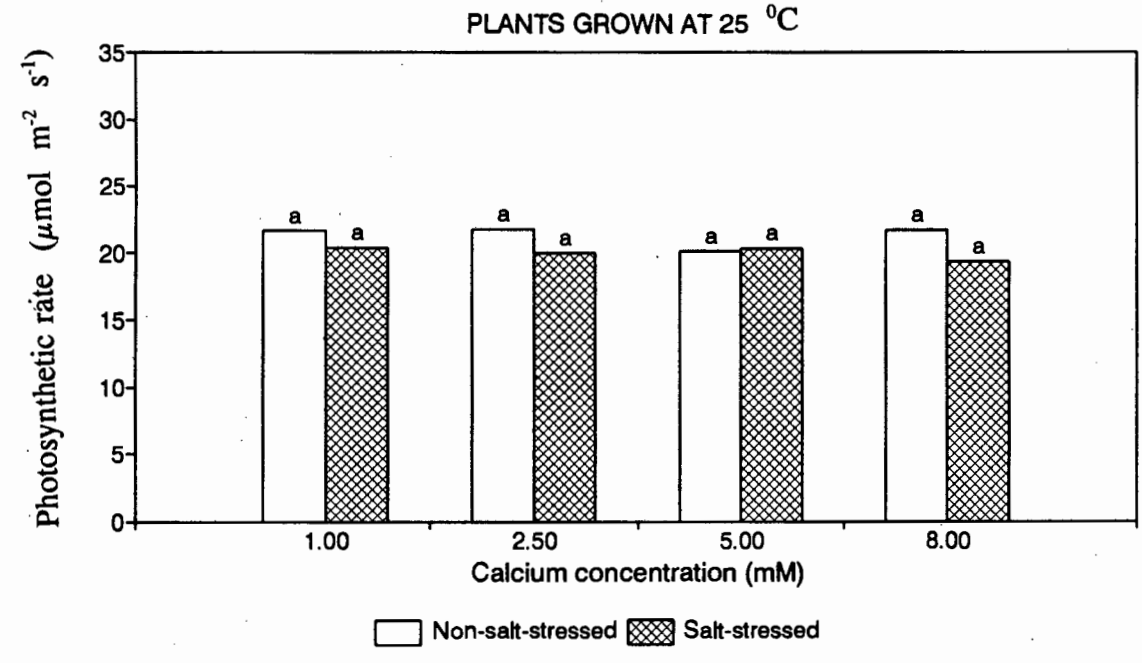
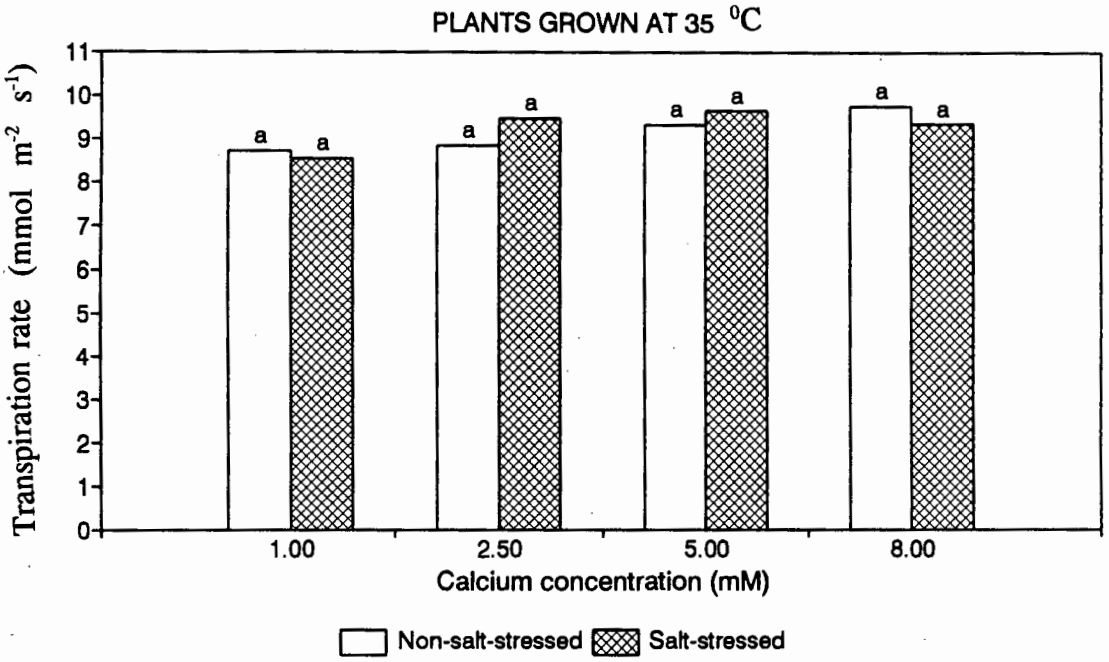


Figure 14. **Effect of a range of calcium concentrations (1 to 8 mM) on the transpiration rates of salt-stressed and non-salt-stressed maize supplied nitrate at 35 ^{0}C and at 25 ^{0}C.** Results are presented as means of 5 replicates for each calcium treatment in both salt-stressed and non-salt-stressed plants. In both salt-stressed and non-salt-stressed plants similar letters show non-significant differences among different calcium treatments ($p > 0.05$, One-way Anova) whereas different letters show significant differences among different calcium treatments ($p < 0.05$, One-way Anova).



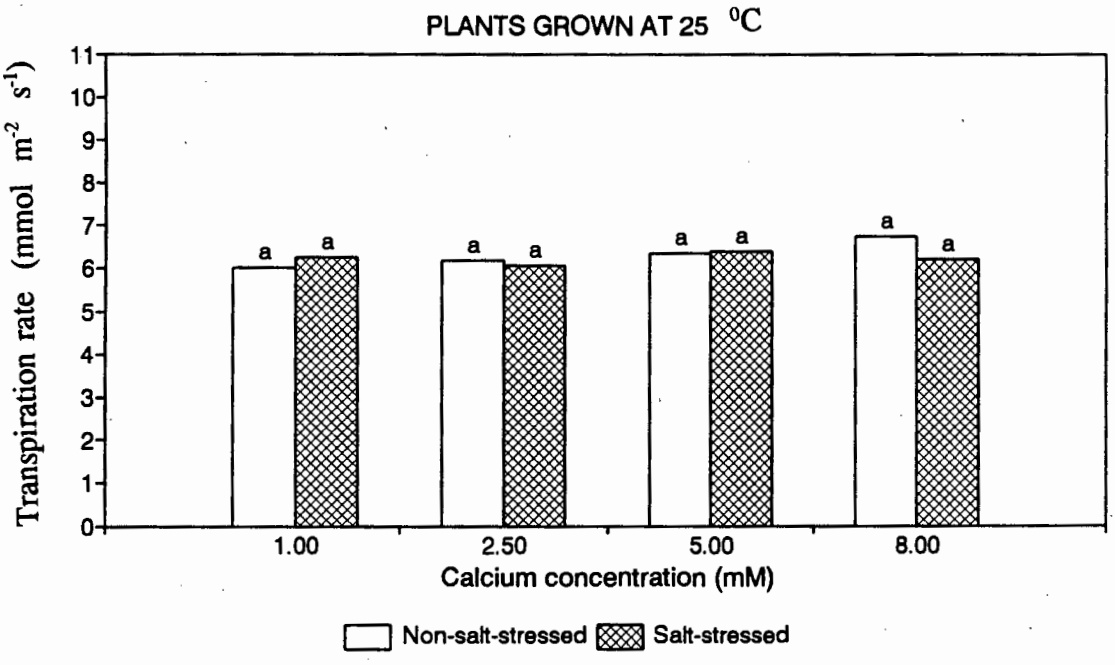
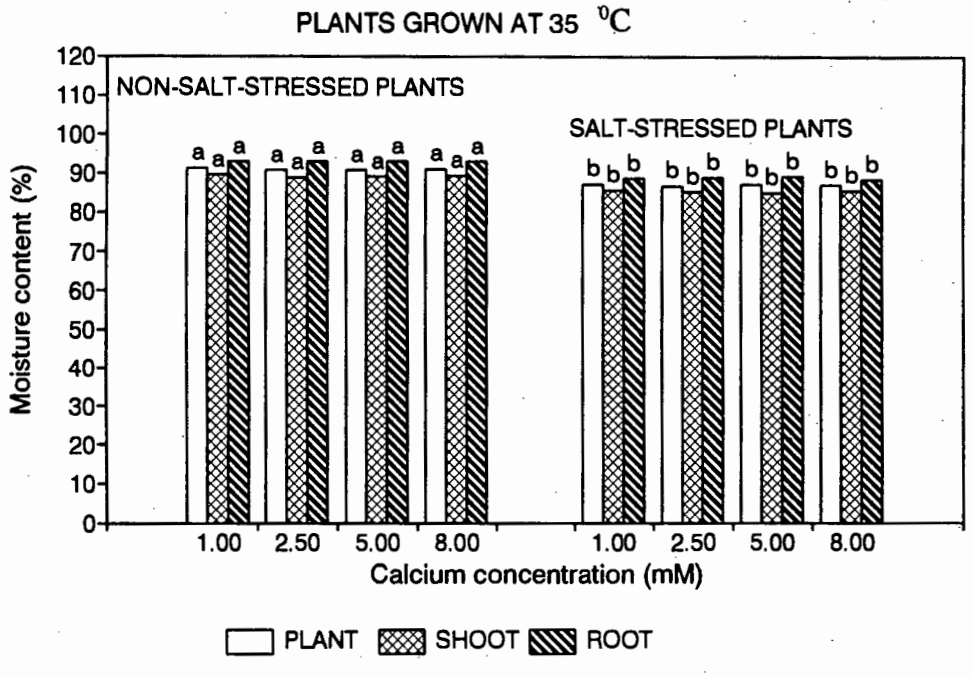


Figure 15. **Effect of a range of calcium concentrations (1 to 8 mM) on the moisture contents of WHOLE PLANTS, SHOOTS and ROOTS of salt-stressed and non-salt-stressed maize supplied nitrate at 35 ^{0}C and at 25 ^{0}C.** Results are presented as means of 16 replicates for each calcium treatment in both salt-stressed and non-salt-stressed plants. In both salt-stressed and non-salt-stressed plants similar letters show non-significant differences among different calcium treatments ($p > 0.05$, One-way Anova) whereas different letters show significant differences among different calcium treatments ($p < 0.05$, One-way Anova).



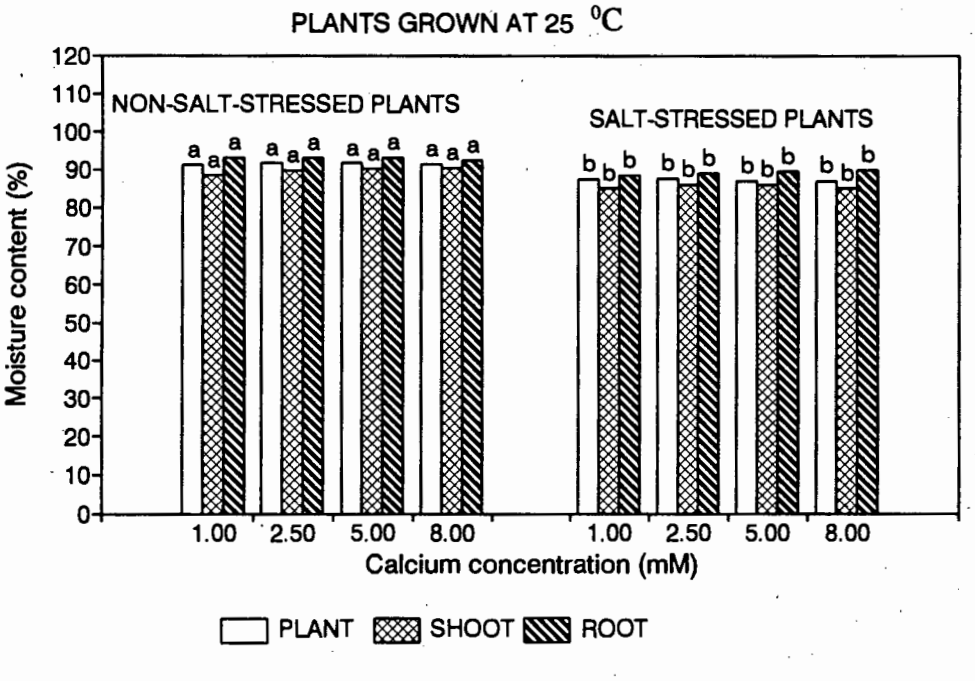
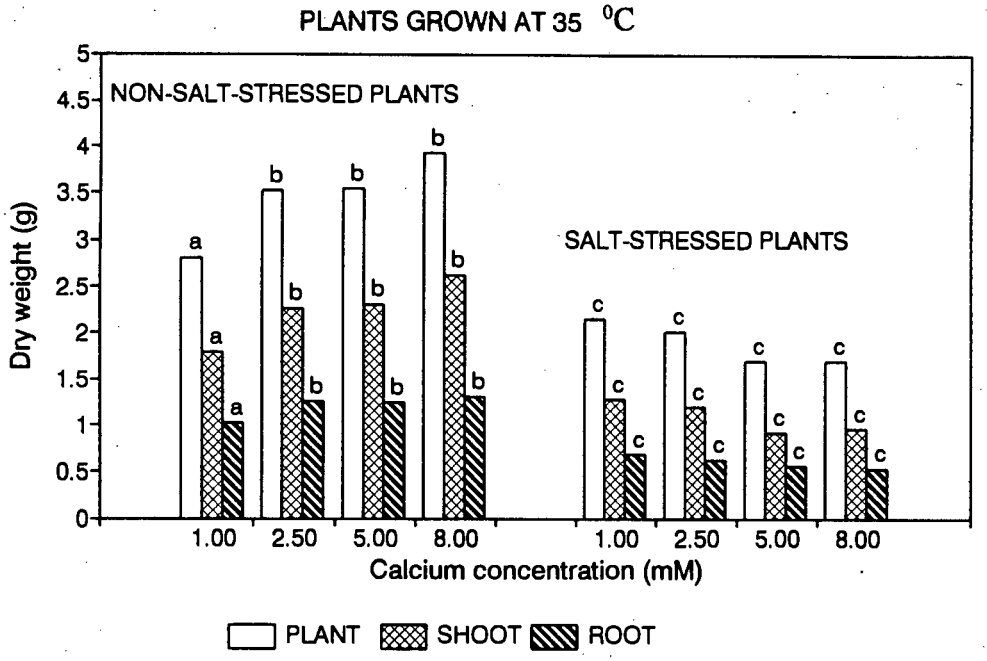
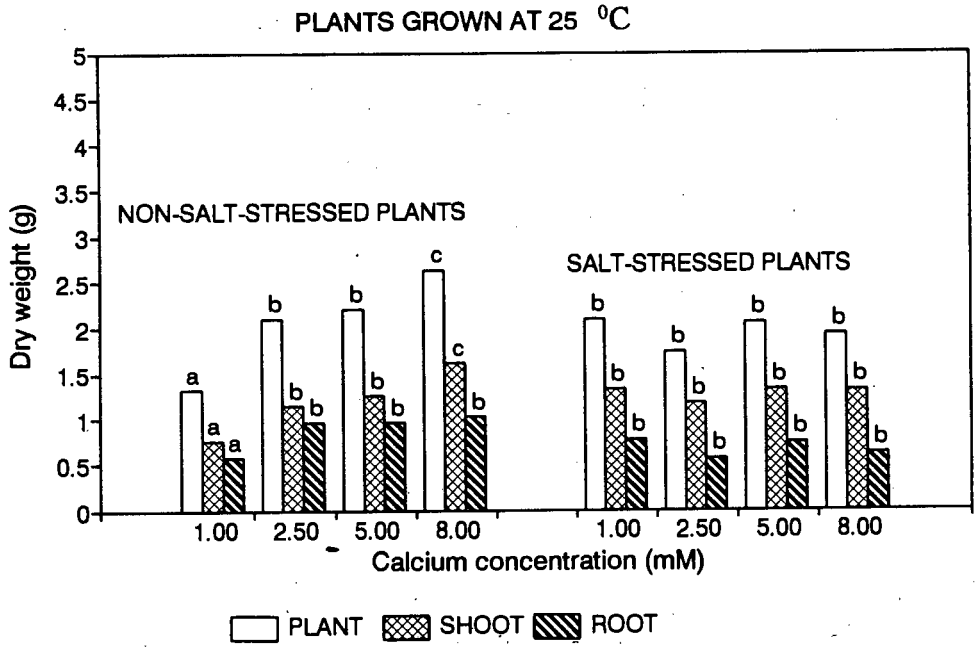


Figure 16. **Effect of a range of calcium concentrations (1 to 8 mM) on the dry weights of WHOLE PLANTS, SHOOTS and ROOTS of salt-stressed and non-salt-stressed maize supplied nitrate at 35 °C and at 25 °C.** Results are presented as means of 16 replicates for each calcium treatment in both salt-stressed and non-salt-stressed plants. In both salt-stressed and non-salt-stressed plants similar letters show non-significant differences among different calcium treatments ($p > 0.05$, One-way Anova) whereas different letters show significant differences among different calcium treatments ($p < 0.05$, One-way Anova).





5.4 THE EFFECT OF A COMBINATION OF DIFFERENT TEMPERATURES (35 ^{0}C AND 25 ^{0}C) AND A RANGE OF CALCIUM CONCENTRATIONS (0.5 TO 5 mM) ON THE RESPONSE OF NITRATE-FED MAIZE TO SALINITY.

5.4.1 Gaseous exchange response

Figures 17 and 18 show the photosynthetic rates and transpiration rates of salt-stressed and non-salt-stressed plants grown at 35 ^{0}C and at 25 ^{0}C in nutrient media supplemented with different calcium concentrations. From these figures it can be seen that the photosynthetic rates and transpiration rates of plants grown at 35 ^{0}C and at 25 ^{0}C did not show a significant response to salinity (Two-way Anova, $p > 0.05$). Photosynthetic rates and transpiration rates of salt-stressed and non-salt-stressed plants grown at either 35 ^{0}C or 25 ^{0}C were not significantly affected by calcium (One-way Anova, $p > 0.05$). For both salt-stressed and non-salt-stressed plants the photosynthetic rates and transpiration rates of maize plants grown at 35 ^{0}C were significantly higher than those of plants grown at 25 ^{0}C (Two-way Anova, $p < 0.05$).

5.4.2 Nitrate uptake response

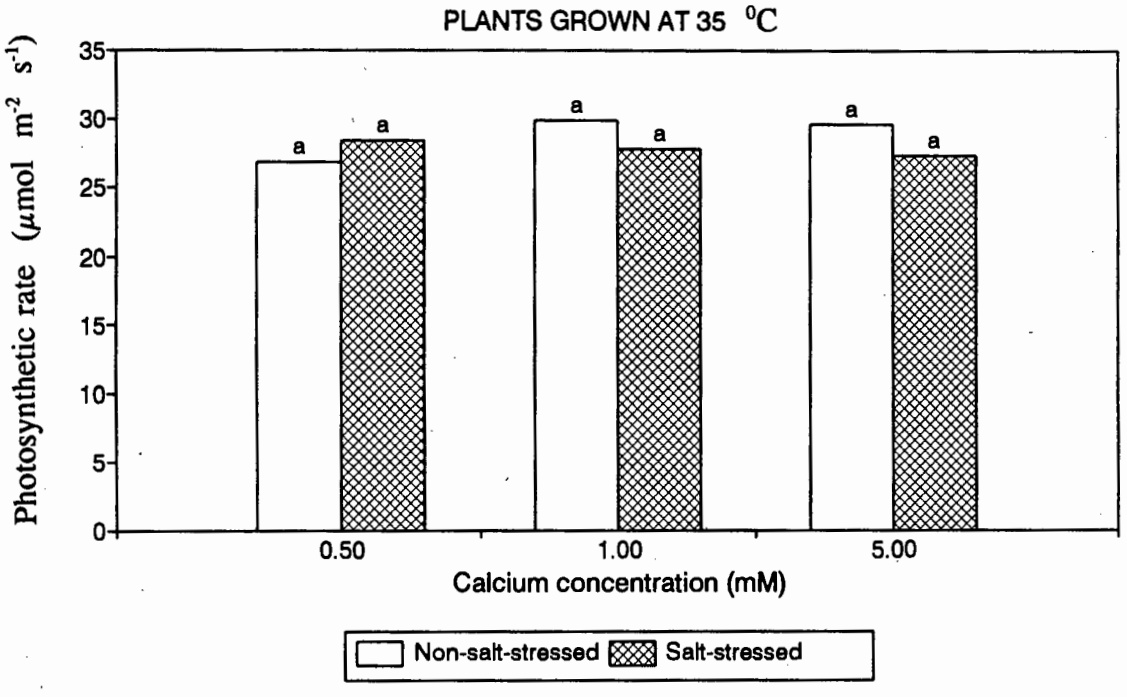
The ^{15}N-labelled nitrate contents of shoot and root of salt-stressed and non-salt-stressed plants grown at 25 ^{0}C in a nutrient medium containing 0.5 mM Ca^{2+} are shown in Figure 19. This figure shows that the ^{15}N-labelled nitrate contents of salt-stressed whole plants, shoots and roots were significantly lower than those of non-salt-stressed plants (t-test, $p < 0.05$).

5.4.3 Growth response

Figure 20 shows the dry weights of whole plants, shoots and roots of salt-stressed and non-salt-stressed plants grown at 35 ^{0}C and at 25 ^{0}C in nutrient media supplemented with different calcium concentrations. Under high temperature conditions (35 ^{0}C), growth of nitrate-fed plants was significantly inhibited by salinity ($p < 0.05$, Two-way Anova). Contrary to these results, when grown at 25 ^{0}C the dry weights of salt-stressed plants were significantly larger than those of non-salt-stressed plants (two-way Anova, $p < 0.05$). Increasing the calcium concentration from 0.5 to 5 mM resulted in a significant increase in growth of non-salt-

stressed plants grown at 35 ^{0}C and at 25 ^{0}C (One-way Anova, $p < 0.05$). On the other hand growth of salt-stressed plants grown at 35 ^{0}C and at 25 ^{0}C did show any significant response to the increments in calcium concentrations (One-way Anova, $p > 0.05$). Under non-saline conditions the dry weights of maize plants grown at 35 ^{0}C were significantly larger than those of plants grown at 25 ^{0}C ($p < 0.05$, Two-way Anova). On the other hand the dry weights of salt-stressed maize plants grown at 35 ^{0}C were not significantly different from those of salt-stressed plants grown at 25 ^{0}C ($p > 0.05$, two-way Anova).

Figure 17. **Effect of a range of low calcium concentrations (0.5 to 5 mM) on the photosynthetic rates of salt-stressed and non-salt-stressed maize supplied nitrate at 35 ^{0}C and at 25 ^{0}C**. Results are presented as means of 5 replicates for each calcium treatment in both salt-stressed and non-salt-stressed plants. In both salt-stressed and non-salt-stressed plants similar letters show non-significant differences among different calcium treatments ($p > 0.05$, One-way Anova) whereas different letters show significant differences among different calcium treatments ($p < 0.05$, One-way Anova).



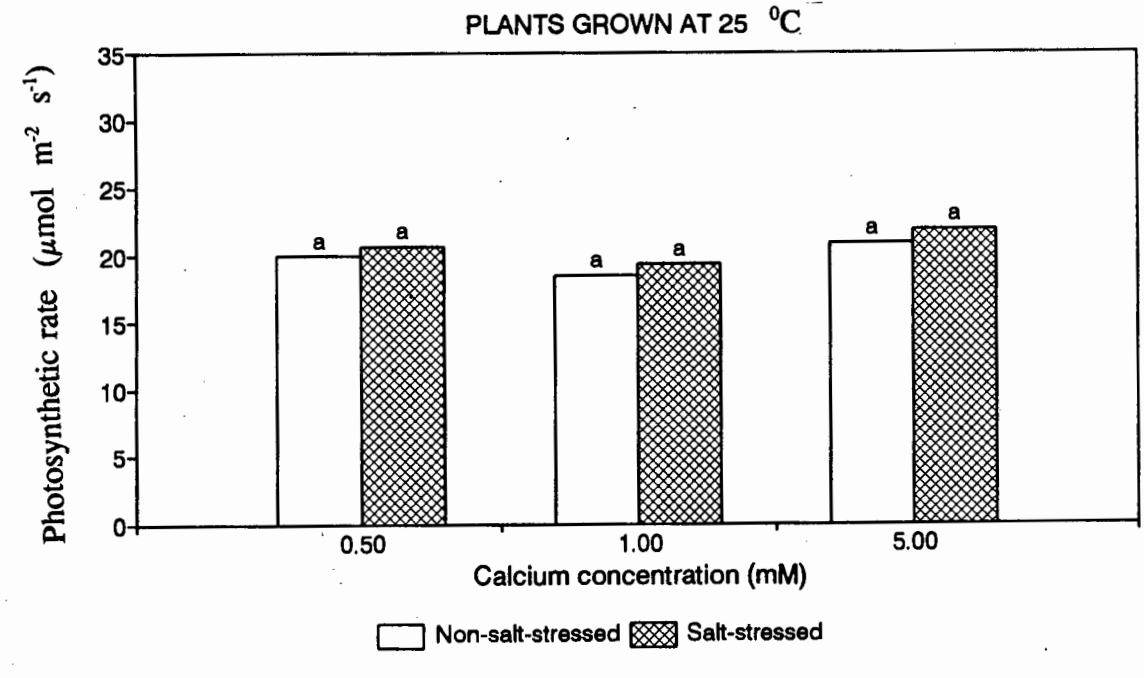
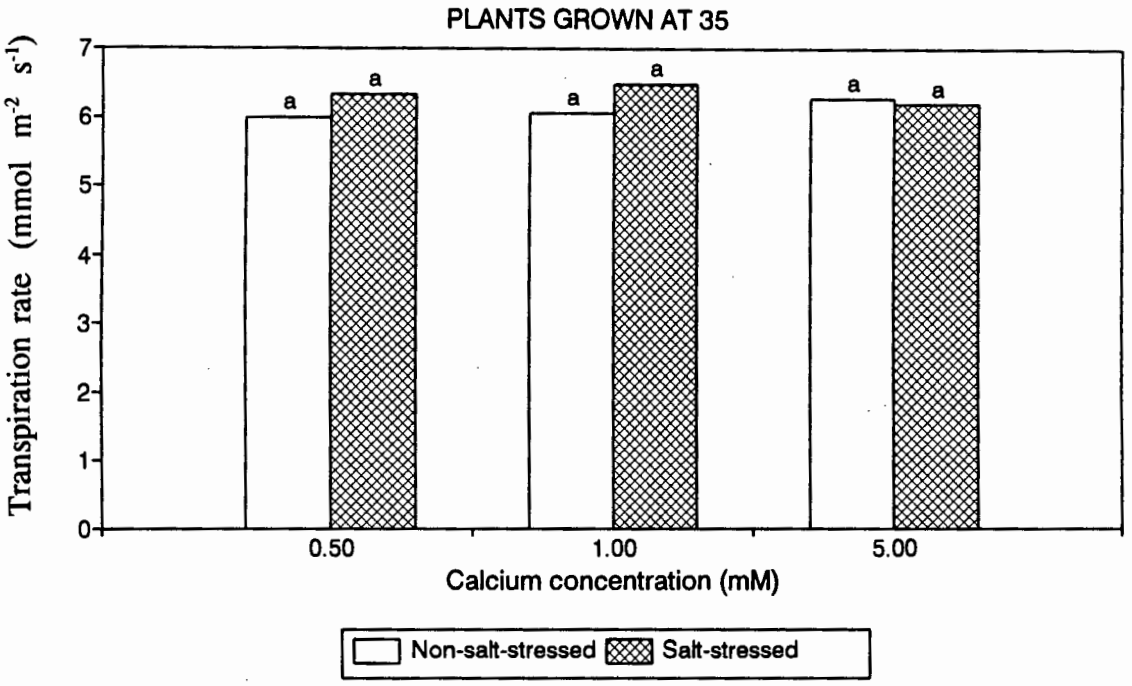


Figure 18. **Effect of a range of low calcium concentrations (0.5 to 5 mM) on the transpiration rates of salt-stressed and non-salt-stressed maize supplied nitrate at 35 ^{0}C and at 25 ^{0}C.** Results are presented as means of 5 replicates for each calcium treatment in both salt-stressed and non-salt-stressed plants. In both salt-stressed and non-salt-stressed plants similar letters show non-significant differences among different calcium treatments ($p > 0.05$, One-way Anova) whereas different letters show significant differences among different calcium treatments ($p < 0.05$, One-way Anova).



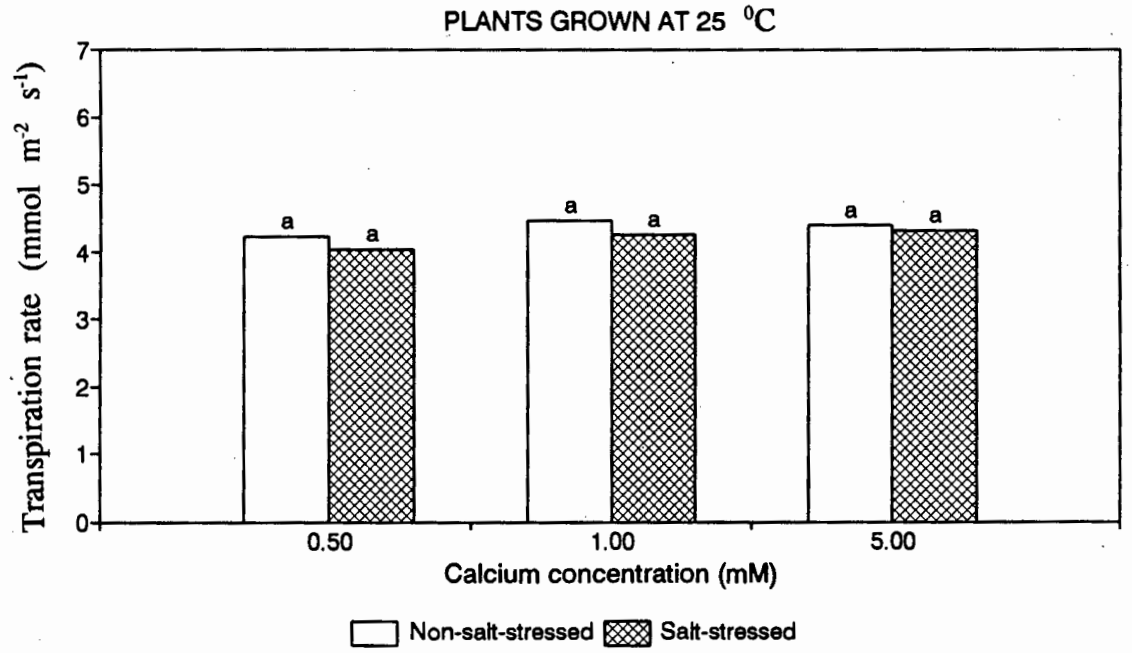


Figure 19. **^{15}N content of WHOLE PLANTS, SHOOTS and ROOTS of nitrate-fed maize grown at 25 ^{0}C in saline and non-saline nutrient media containing a low concentrations of calcium (0.5 mM Ca^{2+}).** Results are presented as means of replicates. Similar letters indicate non-significant ($p > 0.05$, t-test) differences between the salt-stressed and non-salt-stressed plants, whereas different letters indicate significant ($p < 0.05$, t-test) differences between these plants.

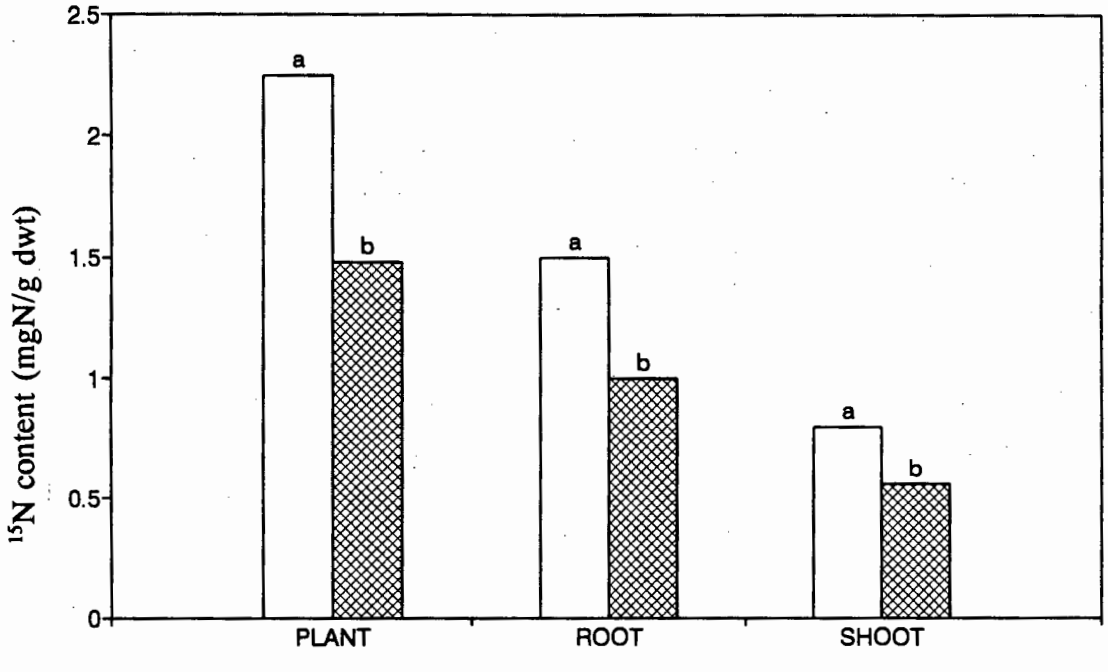
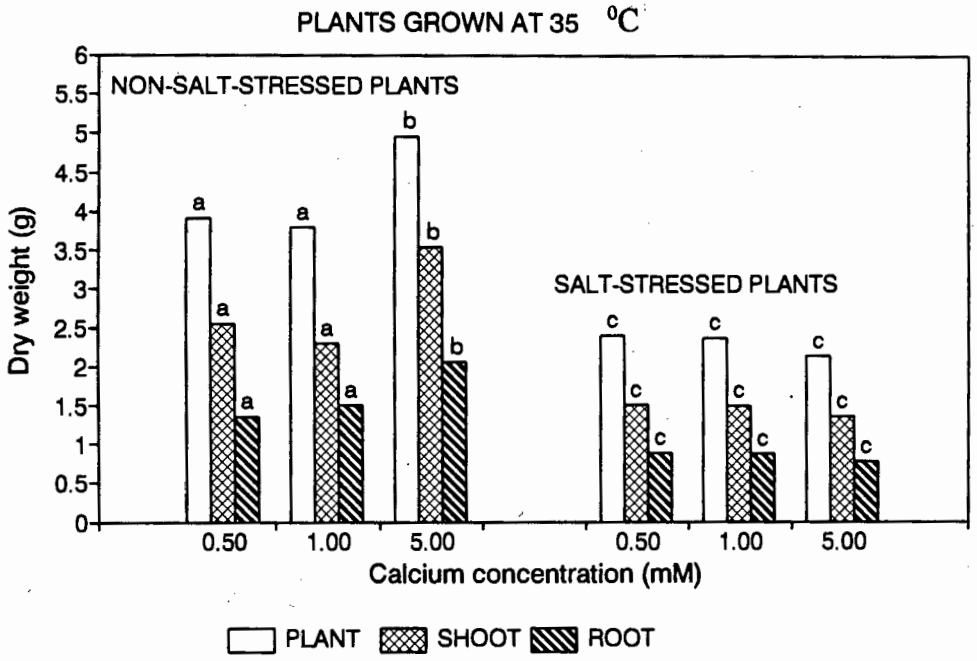
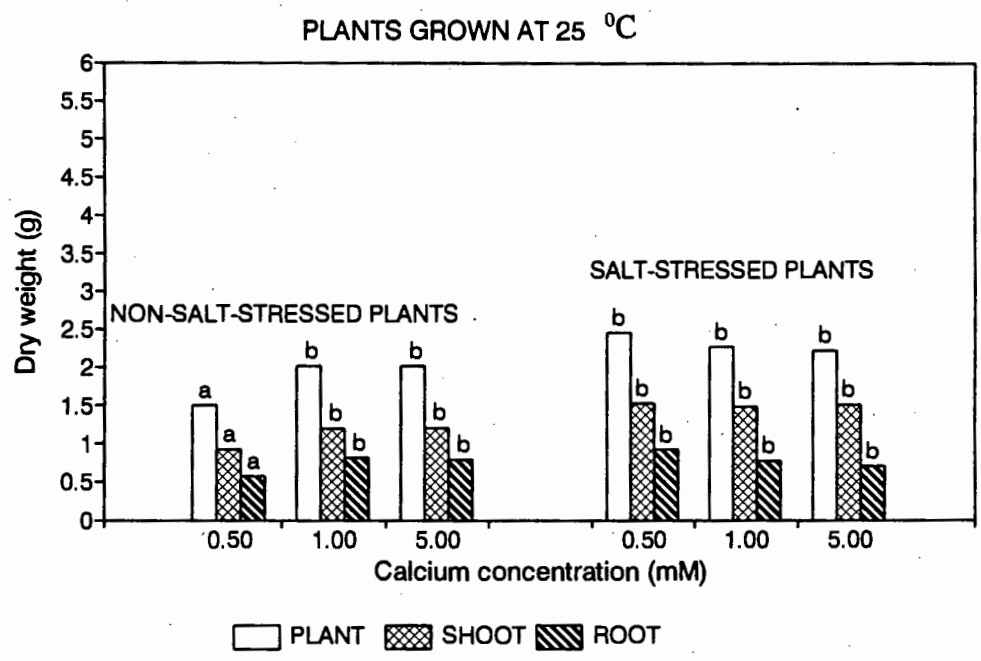


Figure 20. **Effect of a range of low calcium concentrations (0.5 to 5 mM) on the dry weights of WHOLE PLANTS, SHOOTS and ROOTS of salt-stressed and non-salt-stressed maize supplied nitrate at 35 ^{0}C and at 25 ^{0}C.** Results are presented as means of 16 replicates for each calcium treatment in both salt-stressed and non-salt-stressed plants. In both salt-stressed and non-salt-stressed plants similar letters show non-significant differences among different calcium treatments ($p > 0.05$, One-way Anova) whereas different letters show significant differences among different calcium treatments ($p < 0.05$, One-way Anova).





5.5 EFFECT OF POTASSIUM ON THE RESPONSE OF NITRATE- AND AMMONIUM-FED MAIZE TO SALINITY

5.5.1 Gaseous exchange response

The photosynthetic rates and transpiration rates of salt-stressed and non-salt-stressed plants grown in ammonium-containing nutrient media supplemented with different potassium concentrations are shown in Figure 21. As can be seen from this figure, the photosynthetic rates and transpiration rates of salt-stressed plants were significantly reduced when compared to those of their non-salt-stressed controls (Two-way Anova, $p < 0.05$). Potassium did not have any significant effect on photosynthetic rates and transpiration rates of either salt-stressed or non-salt-stressed plants (One-way Anova, $p > 0.05$).

5.5.2 Moisture content response

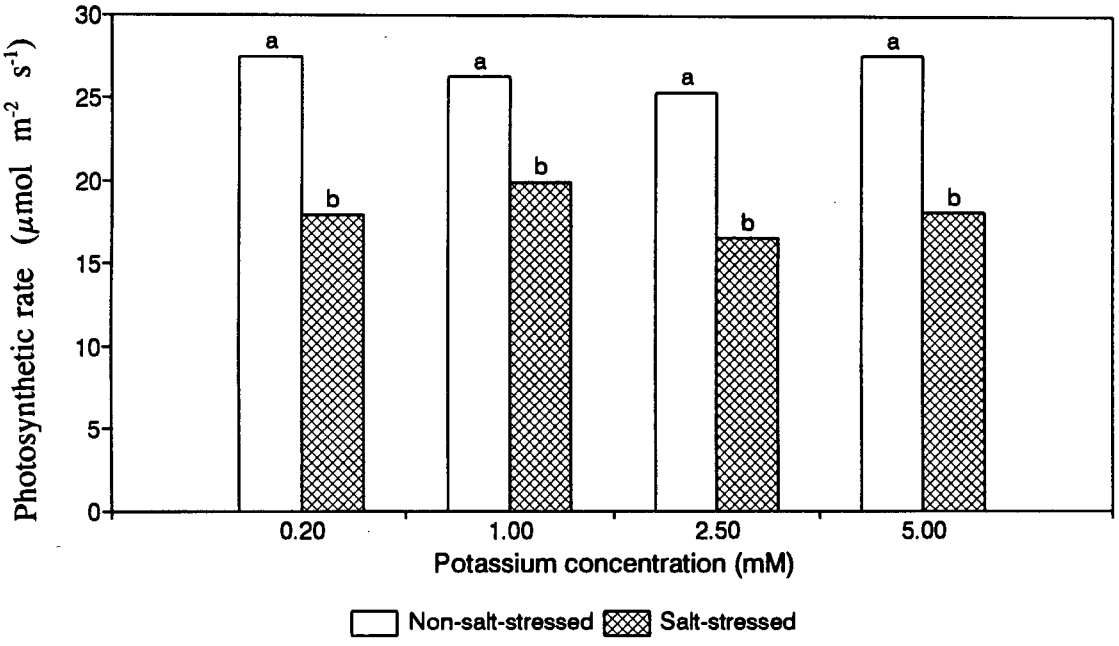
Figure 22 shows the moisture contents of shoots and roots of salt-stressed and non-salt-stressed plants grown in nitrate- and in ammonium-containing nutrient media supplemented with different concentrations of potassium. This figure shows that in both nitrate- and ammonium-fed plants the moisture contents of salt-stressed shoots and roots were significantly reduced when compared to those of their non-salt-stressed controls (Two-way Anova, $p < 0.05$). In both nitrate- and ammonium-fed plants moisture contents of salt-stressed and non-salt-stressed shoots and roots were not significantly affected by potassium (One-way Anova, $p > 0.05$).

5.5.3 Growth response

Dry weights of shoots and roots of ammonium- and nitrate-fed plants grown in saline and non-saline nutrient media supplemented with different potassium concentrations are shown in Figure 23. From this figure it can be seen that in both nitrate-and ammonium-fed plants the dry weights of salt-stressed shoots and salt-stressed roots were significantly lower than those of non-salt-stressed plants (Two-way Anova, $p < 0.05$). Dry weights of shoots of salt-stressed and non-salt-stressed plants increased with an increase in potassium concentration. At a concentration of 1 mM KCl and above the dry weights of salt-stressed plants were the

same. Under non-saline conditions the dry weights of nitrate-fed plants did not significantly increase with an increase in potassium concentration from 2.5 to 5 mM (One-way Anova, $p > 0.05$) whereas those of ammonium-fed plants significantly increased with an increase in potassium concentration from 2.5 to 5 mM (One-way Anova, $p < 0.05$). When grown in non-saline nutrient media nitrate-fed plants did non grow significantly larger than ammonium-fed plants (Two-way Anova, $p > 0.05$) whereas under saline conditions nitrate-fed plants grew significantly larger than ammonium-fed plants (Two-way Anova, $p < 0.05$).

Figure 21. **Effect of potassium concentration on the photosynthetic rates and transpiration rates of salt-stressed and non-salt-stressed maize supplied AMMONIUM.** Results are presented as means of 5 replicates for each calcium treatment in both salt-stressed and non-salt-stressed plants. In both salt-stressed and non-salt-stressed plants similar letters show non-significant differences among different potassium treatments ($p > 0.05$, One-way Anova) whereas different letters show significant differences among different potassium treatments ($p < 0.05$, One-way Anova).



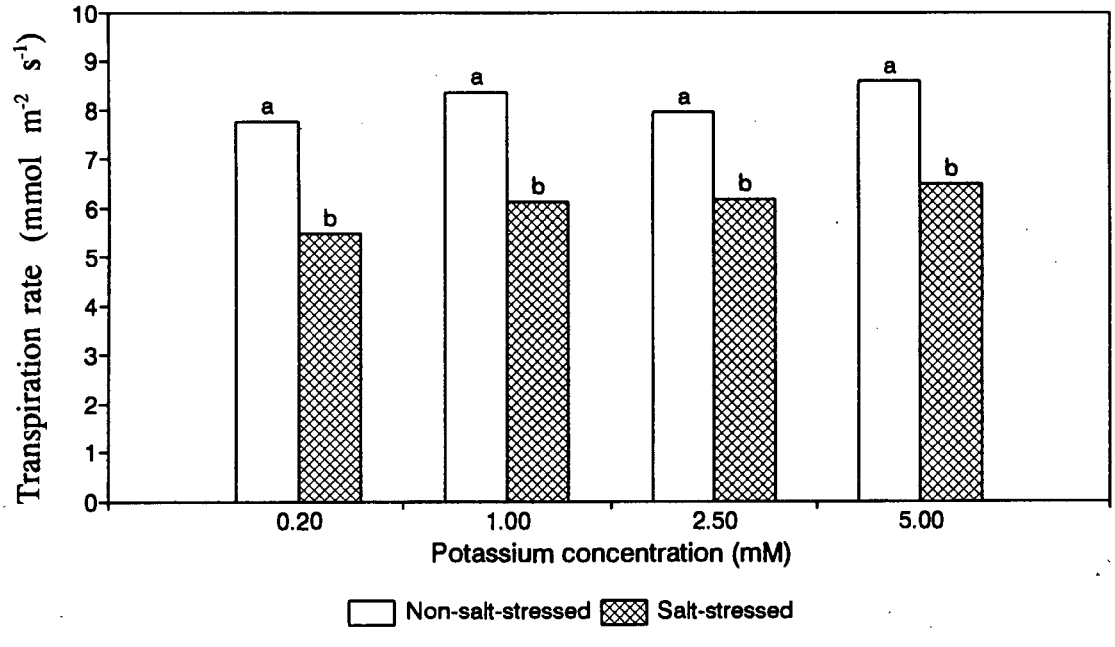
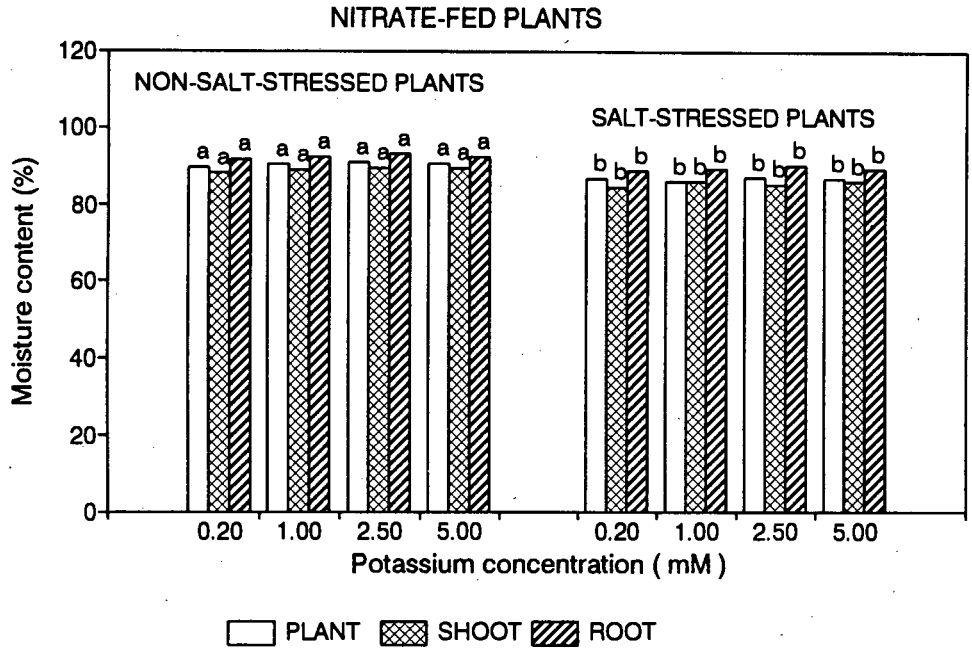


Figure 22. **Effect of potassium concentration on the moisture contents of WHOLE PLANTS, SHOOTS and ROOTS of salt-stressed and non-salt-stressed maize supplied NITRATE or AMMONIUM.** Results are presented as means of 16 replicates for each potassium treatment in both salt-stressed and non-salt-stressed plants. In both salt-stressed and non-salt-stressed plants similar letters show non-significant differences among different potassium treatments ($p > 0.05$, One-way Anova) whereas different letters show significant differences among different potassium treatments ($p < 0.05$, One-way Anova).



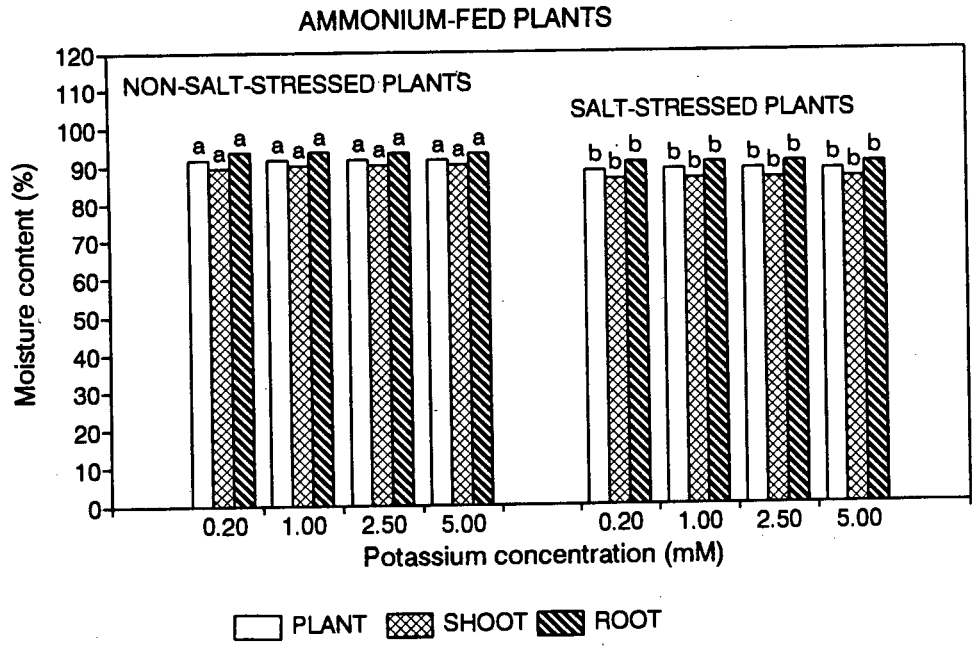
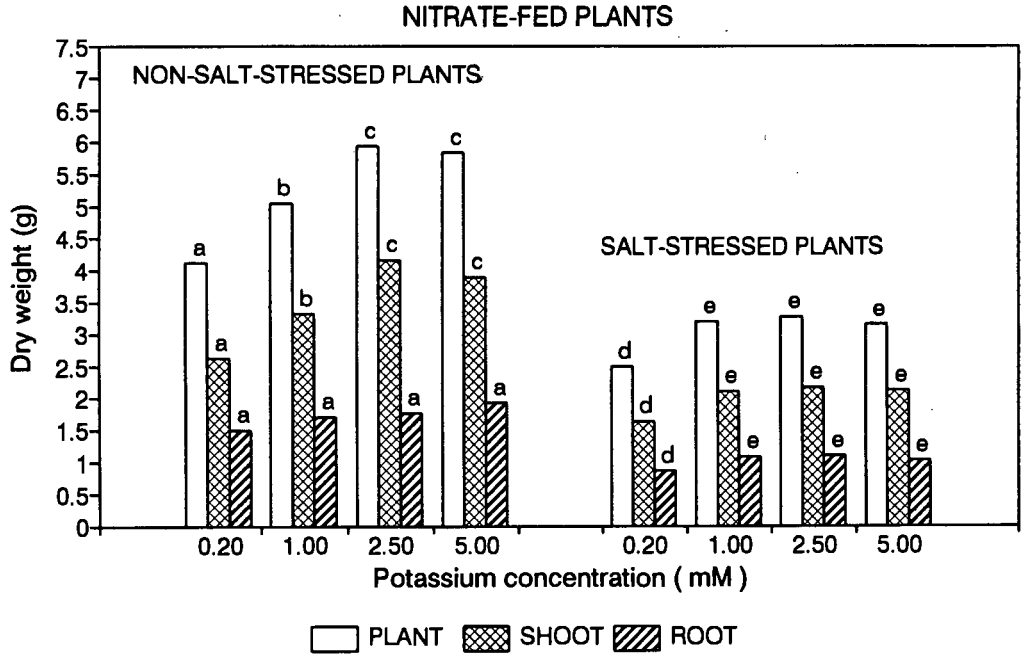


Figure 23. **Effect of potassium concentration on the dry weights of WHOLE PLANTS, SHOOTS and ROOTS of salt-stressed and non-salt-stressed maize supplied NITRATE or AMMONIUM.** Results are presented as means of 16 replicates for each potassium treatment in both salt-stressed and non-salt-stressed plants. In both salt-stressed and non-salt-stressed plants similar letters show non-significant differences among different potassium treatments ($p > 0.05$, One-way Anova) whereas different letters show significant differences among different potassium treatments ($p < 0.05$, One-way Anova).



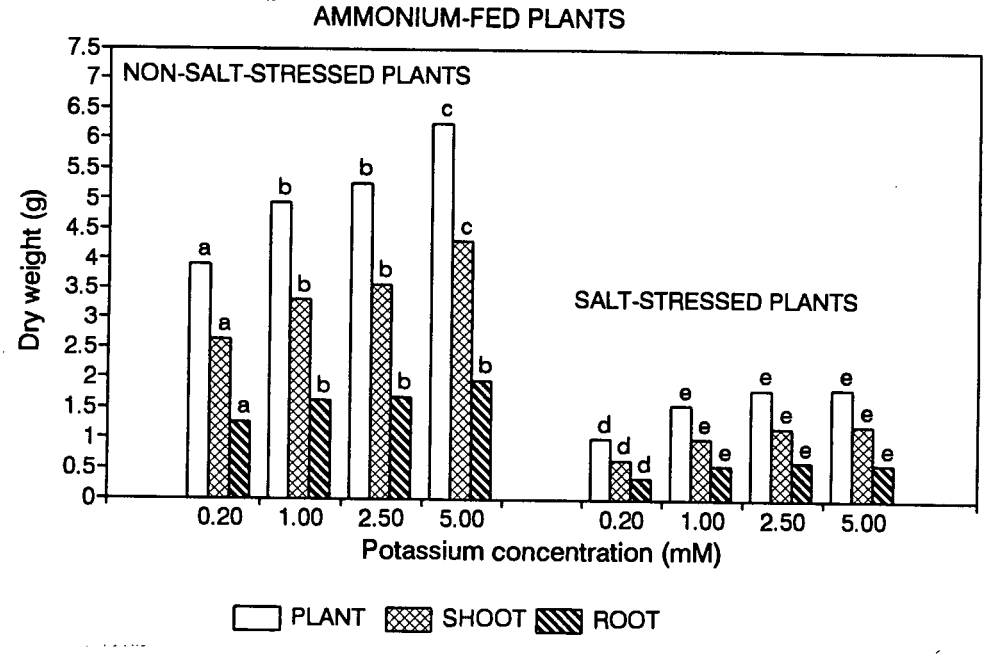
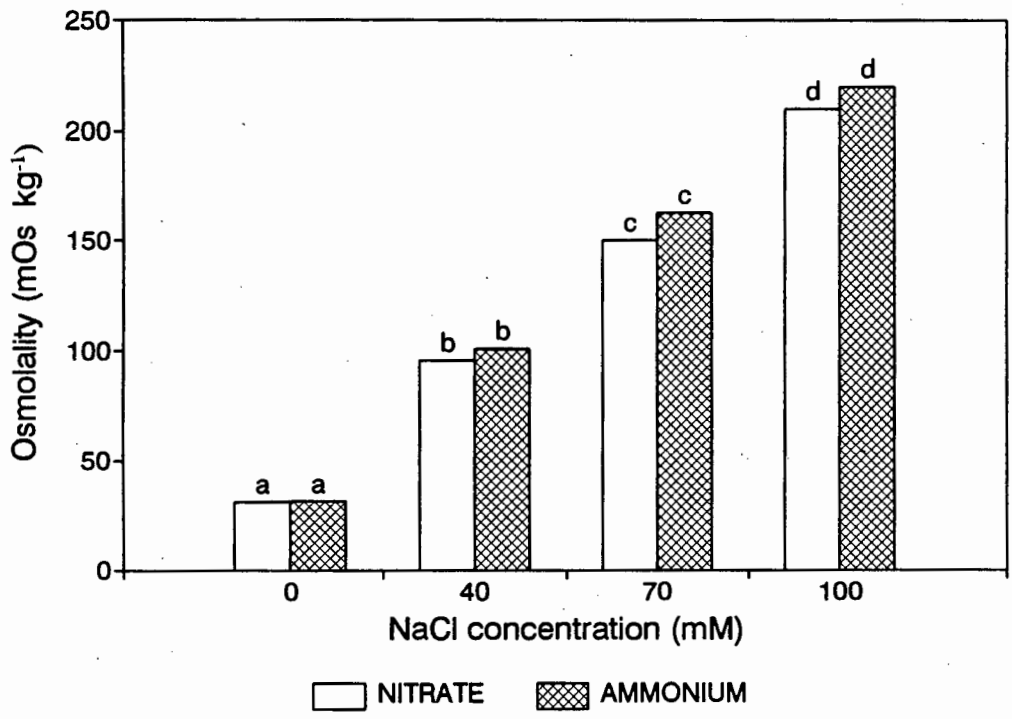


Figure 24. Effect of NaCl concentration on the osmolality of ammonium- and nitrate-containing nutrient solutions. Results are presented as means of four replicates for each NaCl treatment in both ammonium- and nitrate-containing nutrient solutions. In both nitrate- and ammonium-containing nutrient solutions similar letters show non-significant differences among different NaCl treatments ($p > 0.05$, One-way Anova) whereas different letters show significant differences among different NaCL treatments ($p < 0.05$, One-way Anova).



CHAPTER 6

DISCUSSION AND CONCLUSIONS

6.1 DIFFERENCES BETWEEN NITRATE- AND AMMONIUM-FED MAIZE PLANTS IN THEIR RESPONSE TO SALINITY.

6.1.1 Growth effects

When grown in non-saline nutrients, dry weights of shoot and root of nitrate- and ammonium-fed plants were not significantly different (Figure 4). These results are different from those of Lewis, Leidi and Lips (1989) and Murphy (1984) who found that when grown under non-saline conditions, ammonium-fed maize plants were perceptibly larger than the nitrate-fed plants.

The dry weight shoot:root ratio of salt-stressed ammonium-fed maize plants was significantly larger than that of nitrate-fed plants, showing the retarding effect of ammonium nutrition on roots of maize (Figure 4). The retarding effect of ammonium nutrition on the root has also been observed in other plant species such as barley (Warnke and Barber, 1973; Klem, 1966; Lewis and Chadwick, 1983) and wheat (Lewis, Fulton and Von Zelewski, 1987), and has been attributed to a pH decrease in the medium around the roots. Unless buffered, the absorption of ammonium brings about a decrease in the pH of the growth medium due to the concomitant release of H^+ ions. In this study the development of ammonium-fed maize was poor even though the pH was maintained at 5.5 ± 0.5.

Under saline conditions, dry weights of shoot and root of nitrate-fed maize plants were significantly larger than those of ammonium-fed plants, a feature which was not observed under non-saline conditions (Figure 4). These results suggest that ammonium-fed plants are more sensitive to salinity stress than their nitrate-fed counterparts. Lewis, Leidi and Lips (1989) also found that nitrate-fed maize plants are more tolerant to salinity than ammonium-fed plants. According to these researchers, the cause of the greater sensitivity of ammonium-fed maize plants to salinity could be ascribed to the following factors.

(i) In maize, nitrate assimilation takes place primarily in the shoot and ammonium

assimilation in the root. As the roots are in immediate contact with the saline containing nutrient medium it is possible that nitrogen assimilation in ammonium-fed plants is dislocated by ionic effects which could not interfere with leaf-based nitrogen assimilation.
(ii) In nitrate-fed plants a malate-nitrate shuttle is operative between shoot and root (Lips et al., 1970). The absence of this shuttle in ammonium-fed plants could bring about ion translocatory problems which are exacerbated by the uptake of NaCl.
(iii) The assimilation of the bulk of nutrient nitrogen in the roots of ammonium-fed plants necessitates the diversion of large quantities of carbon to the root to provide the carbon skeletons of the products of nitrogen assimilation. It is possible that this carbon metabolism is inhibited by the presence of high concentrations of sodium and chloride ions in the root.

In this study no experimentation was done to ascertain whether or not specific ion toxicity was also involved in growth reduction of salt-stressed maize. However, apart from reduced growth, ammonium-fed plants also showed severe leaf damage, a feature which was not observed in nitrate-fed plants. Leaf damage in some salt-stressed plant species such as avocado (Bingham et al., 1968) and grapevine (Bernstein et al., 1969) has been associated with accumulation of chloride ions in leaves. Having the same charges, nitrate and chloride ions have been found to compete for the same entry port into root cells. It has also been found that high concentrations of nitrate in the growth medium are able to competitively reduce the amount of chloride ions entering root cells (Smith, 1973; Smith and Fox, 1977). Being cations, ammonium ions are unlikely to compete with chloride ions for the same transport system, hence cannot inhibit the uptake of these ions. This could mean that under saline conditions more chloride ions would accumulate in ammonium-fed plants than in nitrate-fed plants. Ammonium-fed plants are most likely to suffer more from specific chloride ion toxicity than nitrate-fed plants and this could be the reason for the severe leaf injury and death observed in ammonium-fed plants under saline conditions. It could be possible that the inability of ammonium ions to suppress the uptake of chloride ions is also a contributory factor to the lower tolerance of ammonium-fed plants to salinity.

Potassium is a major plant nutrient which plays a significant role in osmoregulation in some species of higher plants (Morgan, 1984; Itoh et al., 1986) and in a variety of metabolic processes such as nitrate uptake (Hewitt, 1966; Lips et al.,1970; Ben-Zioni et al., 1971) and protein synthesis (Hsiao and Läuchli, 1986). The significant reduction in growth of both

ammonium- and nitrate-fed plants (though ammonium-fed plants were more affected than nitrate-fed plants) by salinity could be ascribed to salinity-induced potassium deficiency. The fact that under saline conditions potassium nutrition was more disturbed by salinity in ammonium-fed maize plants than in nitrate-fed plants (Figure 7) could also account for less tolerance of ammonium-fed plants to salinity.

When working with wheat and maize Lewis, Leidi and Lips (1989) found the salinity effect on the growth of these plant species to be more noticeable in the shoots than in the roots. When working with barley plants Delane et al. (1982) found results similar to these. Shoot:root ratio results in this study showed that in maize plants fed both nitrogen sources the salinity effect on growth of shoots was not significantly different from that on growth of roots (Figure 4).

6.1.2 **Gaseous exchange effects**

Transpiration rates of nitrate-fed maize plants did not show any significant response to salinity whereas that of ammonium-fed plants were significantly reduced by salinity (Figure 5). These results are closely related to those of Lewis, Leidi and Lips (1989) who found transpiration rate of nitrate-fed maize to be increased by salinity and that of ammonium-fed maize plants to be reduced. Since the addition of salt to the nutrient solution lowers its osmotic potential, the salt stress will expose the plants to secondary osmotic stress or the so called "physiological drought stress" (Munns and Passioura, 1984). Reduction in transpiration rate could be a strategy adopted by ammonium-fed plants to cope with salinity-induced water stress.

Potassium ion can also be used as an osmoticum and can therefore play a major role in maintaining cell turgor under water stress conditions (Levitt, 1980, Wyn Jones, 1981). Under saline conditions potassium contents in shoot and root of nitrate-fed plants was significantly more than those of ammonium-fed plants (Figure 7). Therefore it could be argued that under saline conditions nitrate-fed maize plants are able to adjust to osmotic stress more than ammonium-fed maize plants. This could be a possible explanation for the difference between ammonium- and nitrate-fed maize plants in transpirational water loss under saline conditions.

It can be expected that when under osmotic stress, plants must increase their water use efficiency (A/E) or reduce the transpiration ratio (E/A), i.e. they must lose the minimum amount of water for the amount of carbon dioxide gained from the atmosphere. Richardson and McCree (1985) demonstrated that reduced carbon assimilation of sorghum plants under salinity was associated with an increase in water use efficiency, which was due to a more severe decrease in water loss than in carbon gain. In contrast to the findings of Richardson and McCree (1985), experimental results of this study showed that the water use efficiencies of nitrate-fed maize plants were not significantly affected by salinity, whereas those of ammonium-fed maize plants were significantly reduced by salinity (Figure 6).

Photosynthetic rates of nitrate-fed plants were also found to be not significantly affected by salinity whereas those of ammonium-fed plants were significantly reduced by salinity (Figure 5). These results are similar to those reported for transpiration rates. The reduction in photosynthetic rates of ammonium-fed plants could have been caused by partial closure of stomata (indicated by reduction in transpiration rate), a feature which was also noted in Mexican corn plants by Hayward and Spurr (1943) almost 50 years ago.

Apart from stomatal limitation, photosynthesis could also be limited by salinity ionic effects. Leaf damage in some salt-stressed plants has been associated with accumulation of chloride and sodium ion in leaves (Bernstein et al., 1969; Bingham et al., 1968), which in turn has been found to be inversely related to photosynthesis (Yeo et al., 1985; Ziska et al., 1990). Considering the observation that leaves of ammonium-fed plants grown in saline nutrient medium showed greater injuries than those of their nitrate-fed counterparts and that ammonium-fed plants showed a significant negative response to salinity which nitrate-fed plants did not, it can be argued that the reduction in the rate of photosynthesis of ammonium-fed plants could also have resulted from accumulation of toxic ions in leaves (indicated by severe leaf injury).

Potassium ions are also regarded as playing an important role in maintaining turgor pressure in guard cells of the stomata, thereby regulating opening and closing of the stomata (Fischer, 1971; Terry and Ulrich, 1973). Under saline conditions the potassium contents of shoots of ammonium-fed maize plants were significantly lower than those of nitrate-fed maize plants (Figure 7). It could also be possible that deficiency of potassium ions in leaves of

ammonium-fed maize plants resulted in improper stomatal opening which could be the cause of the significant reduction in photosynthetic rate of these plants. This argument could be more valid if stomatal control in maize plants is potassium specific.

6.1.3 **Effects of nitrate and ammonium on potassium uptake**

In this study it was found that under both saline and non-saline conditions, potassium contents of shoots and roots of ammonium-fed maize were significantly lower than those of nitrate-fed maize, suggesting that ammonium is an inhibitor of potassium uptake (Figure 7). Inhibition of potassium uptake by ammonium has also been found in other plant species such as tomato (Ajayi, Maynard and Baker, 1970; Barker, Maynard, and Lachman, 1967; Kirkby and Mengel, 1967); tobacco (Scherer, Mackrown and Leggett, 1984); barley (Bloom and Finazzo, 1986) and also in maize (Rufty, Jackson and Raper, 1982). Contrary to these results, other studies indicated that ammonium does not significantly depress potassium uptake (Cox and Reisenauer, 1973, 1977; Blair, Miller and Mitchell, 1970). Scherer et al. (1984) suggested that the lack of agreement in studies of potassium-ammonium interaction may be the consequence of different experimental conditions (solution nutrient composition and pH, plant species and plant developmental stage).

In this study details of the mechanism by which ammonium reduced potassium contents in roots and shoots were not explored. However, decrease in potassium content could occur if either the influx of this ion was decreased or efflux was increased, or both. Dean-Drummond and Glass (1982) found that in barley, ammonium did not affect potassium efflux but strongly inhibited its influx resulting in a marked decrease in its net uptake. It has been suggested that ammonium inhibition of potassium influx could result from (i) direct competition of ammonium with potassium for a common transport system (Bange, Tromp and Henkes, 1965) and (ii) ammonium-caused changes in membrane potential and pH (Scherer et al, 1984). On the other hand, efflux of potassium in ammonium-fed plants is also linked to ammonium-caused changes in membrane potential. Since both ammonium and potassium are cations, influx of ammonium ions in the cytoplasm will be followed by efflux of potassium ions so that there could be a balance between cations and anions in the cytoplasm (Minnotti, Williams and Jackson, 1969).

6.2 EFFECTS OF NaCl ON VARIOUS PHYSIOLOGICAL CHARACTERISTICS OF MAIZE.

6.2.1. Gaseous Exchange effects

Photosynthetic and transpiration rates of nitrate-fed plants were not affected by salinity (Figures 5, 8, 9, 13, 14, 17 and 18). These results are in agreement with the findings of other researchers who found no significant effect of salinity on photosynthetic rates of other plant species such as maize (Lewis, Leidi and Lips, 1989), sugarbeet (Terry and Waldron, 1986), spinach (Robinson, Downton and Millhouse, 1983) and barley (Munns et. al, 1982). Contrary to the above reported results, the photosynthetic and transpiration rates of ammonium-fed maize were significantly reduced by salinity (Figures 5, 8, 9 and 21). These results also support the findings of other researchers who found salinity-induced reductions in photosynthetic rates of other plants species such as wheat (Rawson, 1986), beans (Helal and Mengel, 1981; Seemann and Sharkey, 1986), maize (Schwarz and Gale, 1984), grapevines (Dowton, 1977) and rice (Yeo, Carpon and Flowers, 1985).

Experimental results of this study as well as those of the above mentioned researchers clearly indicate that there is no consensus on whether reduced photosynthesis is one of the manifestations of salinity toxicity. The disparity between these two views could be ascribed to the differences in (i) sensitivities of the plant species to salinity, (ii) levels of salinity used and (iii) environmental conditions under which studies were conducted. Results of this study suggest that, in addition to the above mentioned factors, the form of nitrogen supplied to the plants could also be a significant factor contributing to sensitivity of photosynthesis of plants to salinity.

6.2.2 Effects on moisture content

In this study the moisture contents of whole plants, shoots and roots were significantly reduced by salinity (Figures 11, 15, and 22). An experiment carried out to determine the osmolalities of nutrient solutions containing different NaCl concentrations showed a significant increase in osmolality of nutrient solution with an increase in NaCl (Fig 24). Since osmolality is directly proportional to osmotic pressure and inversely proportional to

osmotic potential, there will be a reduction in osmotic potential of nutrient solution with an increase in NaCl concentration. Because of this decrease in osmotic potential of salt-containing nutrient solution, plants growing in these saline solutions will be subjected to an osmotic dehydration. This osmotic dehydration could be the cause of the reduction in growth and moisture contents of salt-stressed shoots and roots. These results suggest that osmotic effect could be one of the major factors causing salinity toxicity in maize. Osmotic effects were also found to be causes of salinity toxicity in other plant species such as wheat (Termaat and Munns, 1986), bean plants (O'Learly, 1975) and sugarbeet (Terry and Waldron, 1986). It is suggested that further research on the water status of salinity-stressed maize should be done in order to confirm the view that osmotic effect is a significant cause of salinity toxicity in this plant.

6.2.3 **Effects on potassium uptake**

Experimental results of this study have shown that for both nitrate and ammonium-fed plants, potassium contents of shoots and roots were significantly reduced by salinity (Figure 7). Reduction in the potassium contents of shoots and roots has also been found in other salt-stressed plant species such as cotton (Cramer et al., 1987; Silberbush and Ben-Asher, 1987), barley (Helal and Mengel, 1979) and wheat (Finck, 1976). Calcium plays an important role in the regulation of membrane permeability to various ions, in particular to organic cations (Van Steveninck, 1965). It has also been reported that removal of calcium from the plasma membrane by EDTA causes leakage of intracellular potassium ions (Weimberg et al., 1987). Furthermore displacement of membrane-associated calcium ions by external sodium ions has been found to be a primary response of barley root cells to salt-stress. It was also found that this removal of calcium ions from plasma membrane induces the loss of intracellular potassium ions in roots (Cramer et al., 1985). It is speculated that sodium ions could have displaced maize root membrane-associated calcium ions thereby disturbing the root membrane integrity of these plants. This disturbance in membrane integrity could have resulted in leakage of potassium ions out of the plant root cells and this could serve as an explanation for the reduction in salt-stressed root and shoot potassium contents.

Low potassium contents in roots and shoots of salt-stressed plants could also result from direct competitive inhibition of potassium uptake by sodium ions (Chow et al., 1990). However, the mechanism through which sodium ions inhibit uptake of potassium ions is not clearly understood. It could be assumed that since sodium and potassium ions have the same charges, they might compete for the same transport systems. This assumption is contradicted by the study of Lazof and Cheeseman (1988) who found that even though high concentrations of sodium ions inhibited the uptake of potassium ions in *Spergularia marina*, the pathway for the uptake of sodium ions was completely independent of that of potassium ions. Bange et al. (1959) and Black (1960) suggested mechanisms for absorption of sodium and potassium ions to be (i) a sodium mechanism where potassium ions can compete when sodium ions are in low concentrations and (ii) a potassium mechanism which is completely independent of competition from sodium ions.

6.2.4 **Effects on nitrate uptake**

The uptake of ^{15}N into the shoots and roots of maize was significantly reduced by salinity of the feeding medium (Figures 10 and 19). These results are in agreement with the findings of other researchers (Helal, Koch and Mengel, 1975; Aslam, Huffaker and Rains, 1984) who reported inhibitory effects of salinity on the uptake and assimilation of ^{15}N-labelled nitrate in barley. In this study no investigation of mechanisms involved in the inhibition of nitrate uptake in salinity-stressed maize was carried out. However, studies done by other researchers (Dean-Drummond and Glass, 1982, Cram and Smith, 1973) have shown that since Cl^- and NO_3^- have the same charges, they compete for the same transport system from the growth medium into the roots. Therefore in saline nutrient medium Cl^- ions inhibit the uptake of NO_3^- ions competitively.

In the malate-nitrate circulation model (Lips et al., 1970) it was proposed that nitrate would migrate from the roots to the leaves via the xylem, with a concomitant synthesis and accumulation of malate in the leaf. The malate would then migrate down to the roots via the phloem accompanied by potassium (as K^+-malate). In the roots malate would then be further converted into bicarbonate (HCO_3^-), which would exchange with soil nitrate. Silberbush and Ben-Asher (1987) suggested that under saline conditions high Na^+ can replace K^+. Therefore nitrate might be transported from roots to the leaves via the xylem accompanied by Na^+, but

sodium-malate will not migrate down to the roots. The immobilization of malate in the shoot will thus reduce the uptake of nitrate by the roots.

6.2.5 **Growth effects**

In this study growth of maize grown at 35 ^{0}C (a temperature required for maximum growth by C_4 plants) was always found to be significantly inhibited by salinity (Figures 4, 12, 16, 20 and 23). Apart from specific ion toxicity, other major potential limitations on the growth of plants in saline environments have been identified as osmotic effects and salinity-induced nutrient deficiency (Bernstein and Hayward, 1958; Maas and Niemand, 1978).
Salinity-induced potassium deficiency appears to be a contributory factor to growth reduction in both nitrate- and ammonium-fed maize. In nitrate-fed plants salinity-induced nitrogen deficiency also appears to be a contributory factor to growth reduction in salt-stressed maize. Even though the uptake of nitrogen in salt-stressed maize fed ammonium was not studied, it is most likely that nitrogen deficiency was also involved in growth reduction of these plants. Uptake of nitrate and ammonium was also identified as a key limiting process of growth of barley seedlings growing in saline environments (Helal, Koch and Mengel, 1975; Huffaker and Rains, 1986). The reduction in moisture content of shoot of salt-stressed maize suggest that osmotic effect could also be a factor contributing to growth reduction, but assessment of other parameters of salt-stressed maize shoots such as water potential and turgor pressure is necessary to confirm this view.

Interesting to note is that at ranges of low calcium concentrations (1 to 8 and 0.5 to 5 mM Ca^{2+}) dry weights of plants grown at 35 ^{0}C were significantly larger those of plants grown at 25 ^{0}C (Figures 16 and 20) whereas there were no significant differences between the dry weights of salt-stressed maize plants grown at 35 ^{0}C and those of salt-stressed plants grown at 25 ^{0}C (Figures 16 and 20). The reason for the difference in growth rate of plants grown at 35 ^{0}C and that of plants grown at 25 ^{0}C could be that being a C_4 plant, maize prefers high temperature conditions in order to photosynthesize maximally (Figures 13 and 17), hence grows faster at higher than at low temperature. Growth of plants grown at 35 ^{0}C was reduced by salinity, whereas that of plants grown at 25 ^{0}C was stimulated by salinity and this could be the reason for the absence of any significant difference in the dry weights of salt-stressed maize plants grown at 35 ^{0}C and those of salt-stressed plants grown at 25 ^{0}C. These

results suggest that the effect of salinity on the growth of maize could be temperature-dependent. Lower temperatures promoted the stimulatory effect of salinity on the growth of maize, whereas at high temperatures the deleterious effects of salinity on maize growth were exacerbated. Reasons for this stimulatory effect of NaCl only at lower temperature conditions are not known. The stimulatory effect of salinity on growth of maize was more apparent in plants which were supplied with lowest calcium concentrations (Figures 16 and 20). These results suggest that when calcium supply was inadequate, sodium replaced calcium hence promoted growth. From Figures 16 and 20 it can also be seen that even when an adequate supply of calcium (5 mM Ca^{2+}) was used in the nutrient media, salt-stressed plants were still equal in size to non-salt-stressed plants. Therefore it could be possible that apart from sparing calcium, the sodium ion plays a significant role as an independent nutrient element. NaCl has been found to disturb calcium nutrition (by inhibiting its uptake) of other plant species such as rice (Grieve and Fujiyama, 1987) and barley (Lynch and Lauchli, 1985). To confirm the view that apart from replacing Ca^{2+}, Na^+ plays a role as an independent nutrient element in maize grown at 25 ^{0}C, it will be necessary to make sure that at 5 mM Ca^{2+}, sodium is not significantly disturbing the nutrition of calcium.

Na^+ has been shown to replace K^+ in other plant species. For example, when working with Italian ryegrass, Hylton et al. (1967) found that increasing Na^+ concentration from 1 to 8 mM lowered critical K^+ levels in the leaf at which K^+ deficiency was apparent. In his study it was also found that Na^+ was only able to replace K^+ when the supply of K^+ was neither inadequate nor over abundant. When working with Rhodes grass, Smith (1973) also found results similar to those of Hylton et al. (1967). The results of these researchers strongly suggested that Na^+ is not able to replace K^+ completely. In this study the stimulatory effect of NaCl on growth was only strongly significant in plants which received the lowest concentrations of calcium, indicating that at these concentrations Na^+ was a successful substitute for calcium.

Ohta, Matoh and Takahashi (1988) found nitrate uptake of *Amaranthus tricolor* seedlings growing in Na^+-deficient culture solution to be stimulated by about 210% within 5 hours by addition of 0.5 mM NaCl in the culture solution. From their Na^+-preloading experiment, intracellular Na^+ was shown to be responsible for the stimulation of NO_3^- uptake. In their previous study, Ohta, Matoh and Takahashi (1987) found nitrate reductase activity of

Amaranthus tricolor seedlings growing in Na^+-deficient culture solution to be promoted by addition of 0.5 mM NaCl in the culture solution. The studies of these researchers suggest a possible role of Na^+ in nitrate uptake and its assimilation in *Amaranthus tricolor* plants.

In this study salt-stressed maize plants grown in nitrate-containing nutrient solutions and supplied with low calcium concentration (0.5 mM) at 25 ^{0}C grew significantly larger than non-salt-stressed plants grown under similar conditions (Figure 20). It was hypothesized that the uptake of nitrate into the roots and shoots of maize plants supplied with low calcium concentration (0.5 mM) at 25 ^{0}C was enhanced by salinity (80 mM NaCl). This enhancement of nitrate uptake by salinity was thought to be the reason for higher growth of salt-stressed plants as compared to non-salt-stressed plants. However, the ^{15}N uptake experiment carried out in this study produced results that are contradictory to our hypothesis. Instead of promoting the uptake of nitrate, NaCl inhibited the uptake of nitrate into the roots and shoots of maize plants (Figure 19). This study has indicated that NaCl did not enhance growth of maize plants supplied with low calcium concentration (0.5 mM) by promoting the uptake of nitrate in these plants. Since our experimental conditions (nutrient solution composition, level of salinity and plant species) were different from those of Ohta, Matoh and Takahashi (1988), the contradiction between our study and that of Ohta et al. (1988) does not seem to be real.

6.3 INVESTIGATION OF CALCIUM AS A POSSIBLE AMELIORATING FACTOR IN SALT TOXICITY.

6.3.1 Gaseous exchange effects

The experimental results of this study showed that in all different ranges of calcium concentrations, photosynthetic and transpiration rates of both salt-stressed and non-salt-stressed maize did not show any significant response to calcium (Figures 8, 9, 13, 14, 17, 18 and 21). Lewis, Leidi and Lips (1989) also did not observe any significant effect of calcium on photosynthetic and transpiration rates of salt-stressed wheat.

6.3.2 **Effects on moisture content**

The moisture contents of salt-stressed and non-salt-stressed shoots and roots were also not significantly affected by calcium (Figures 11, 15 and 22). It could then be argued that calcium was unable to mitigate the osmotic effect in salinity-stressed plants. Nakamura et al (1990) also found that high external levels (8 mM) Ca^{2+} could overcome the ion-specific effects but not could not offset the osmotic effects of salinity on root elongation of mung bean.

6.3.3 **Effects on ^{15}N uptake**

Calcium was also found to have no beneficial effect on uptake of ^{15}N-labelled nitrate (Figure 10). These results do not support the findings of other researchers (Ward, Aslam and Huffaker, 1986; Huffaker and Rains, 1986), who found supplementation of calcium in saline medium to promote nitrate uptake and assimilation in barley seedlings. These researchers worked with range of low calcium concentrations (0.5 to 5 mM) whereas in this study a range of high calcium concentrations (2.5 to 8 mM) was used. The contradiction between the results of this study and those found by the above mentioned researchers could be ascribed to the difference in the range of calcium concentrations used. This could mean that calcium is only beneficial to the uptake of nitrate when supplied in lower concentrations to plants growing in saline environments.

6.3.4 **Growth effects**

6.3.4.1 Effect of a range of calcium concentrations (2.5 to 12 mM)

Increasing the calcium concentration from 2.5 to 12 mM did not result in any ameliorative effect on the growth of salt-stressed maize (Figure 12). These results are not in agreement with those of Cramer at al (1990) who found high concentration of calcium (10 mM) supplemented into the saline (125 mM NaCl) nutrient medium to improve growth of barley. At the highest calcium concentration (12 mM) growth of salt-stressed plants were significantly reduced when compared with those of salt-stressed plants grown at lower calcium concentrations. High concentrations of calcium (20 mM) were also found to inhibit cell

elongation in *Avena sativa* (Cleland and Rayle, 1977; Cooil and Bonner, 1957). In this study the highest calcium concentration (12 mM) could have inhibited cell elongation in maize, and this together with additional deleterious effects of salinity could be the cause of the reduction in growth of salt-stressed plants grown in 12 mM Ca2+. It could also be speculated that at the highest calcium concentration, the osmotic effect of salinity was exacerbated by the high Ca^{2+} ion concentration in the nutrient solution. This increase in osmotic effect could also be the cause of growth reduction in salt-stressed plants grown in 12 mM Ca^{2+}.

6.3.4.2 Effect of a range of calcium concentrations (1 to 8 mM)

Increasing the calcium concentration from 1 to 8 mM also did not result in any beneficial effect on the growth of salt-stressed maize grown at either 35 ^{0}C or 25 ^{0}C (Figure 16). These results are contrary to the finding of Nakamura et al (1990) who found a significant increase in root growth of salt-stressed mung bean plants by increasing the calcium in the nutrient medium from 1 to 8 mM. When working with the same range of calcium concentration, Ayoub (1974) showed that in cool seasons calcium caused a competitive inhibition of sodium uptake and translocation which resulted in improved growth of salt-stressed bean (*Phaseolus vulgaris*) plants whereas in warm seasons calcium did not show any ameliorative effect on the growth of these plants. These results suggested that the ability of calcium to enhance salinity-tolerance in *P. vulgaris* is a function of temperature. The experimental results of our study showed that in both high and low temperature conditions, calcium was not beneficial to the growth of salt-stressed maize. In contrast to these results calcium was found to be significantly beneficial to the growth of non-salt-stressed plants indicating a higher optimal concentration of calcium for maize than that used in conventional feeding solutions.

6.3.4.3 Effect of a range of calcium concentrations (0.5 to 5 mM)

Even at this range of calcium concentration, calcium did not show any ameliorative effect on the growth of salt-stressed maize grown at either 35 ^{0}C or 25 ^{0}C. Contrary to these results are the findings of other researchers who found the ameliorative effect of low concentrations of calcium (3 mM) on the growth of other salt-stressed plant species such as barley (Ward, Aslam and Huffaker, 1986) and bean (La Haye and Epstein, 1969; La Haye and Epstein, 1971).

Generally this study showed that calcium had no beneficial effect on the growth of salt-stressed maize grown in the above mentioned calcium or temperature regimes. These results suggest that even though the notion that calcium is a useful tool in promoting growth of plants under saline conditions is well documented (Cramer et al., 1990; Hyder and Greenway, 1965; Kent and Läuchli, 1985; La Haye and Epstein, 1971; Nakamura et al., 1990), it must not be generalized since the effects of calcium might depend on the variety of plant species used, the level of salinity and the growth conditions.

6.4 INVESTIGATION OF POTASSIUM AS A POSSIBLE AMELIORATING FACTOR IN SALT TOXICITY

6.4.1 Gaseous exchange effects

In this study potassium was not found to have any significant effect on the photosynthetic rates and transpiration rates of either salt-stressed or non-salt-stressed plants. Similar results were found for moisture contents of shoots and roots (Figures 21 and 22).

6.4.2 Growth effects

Experimental results of this study showed an ameliorative effect of potassium (1 mM K^+) on the growth of salt-stressed maize supplied nitrate or ammonium (Figure 23). These results are in agreement with those of Helal, Koch and Mengel (1975) who found that additions of potassium concentrations (5 and 10 mM) to the saline (80 mM NaCl) nutrient medium improved the growth of barley plants. However, it should be noted that in this study the ameliorative effect of potassium concentration was also apparent in non-salt-stressed plants. These results suggest that the importance of potassium in maize growth is not only specific to plants growing in saline environments. Mechanisms by which potassium improves salinity tolerance in other plant species have been identified as its ability to enhance nitrate metabolism (Helal, Koch and Mengel, 1975) and to reduce uptake of Na+ ions competitively (Finck, 1976; Huffaker and Wallace, 1960). In this study nitrate uptake appears to be a major limitation on growth of salt-stressed plants (Figures 10 and 19). Therefore it is suggested that potassium improved the growth of salt-stressed maize by minimizing the deleterious effect of salinity on nitrate uptake. Potassium is also important in nitrogen

metabolism in non-salt-stressed plants (Lips et al., 1970). It is most likely that potassium concentration increased the growth of non-salt-stressed maize by improving nitrogen assimilation in these plants.

Increasing the potassium concentration from 2.5 to 5 mM resulted in a significant increase in growth of ammonium-fed plants but not that of nitrate-fed plants indicating a higher optimal concentration of potassium for ammonium-fed maize than that used in conventional feeding solution (Figure 23). When studying the effects of potassium concentrations (0.1 to 5 mM) on various physiological characteristics of ammonium- and nitrate-fed wheat, Lips et al. (1990) found biomass production of ammonium-fed plants to be more dependent on the concentration of K^+ in the nutrient medium than nitrate-fed plants. The K^+-shuttle in plants, which operates in nitrate-fed plants and not in ammonium-fed plants, was suggested as an explanation for the large dependence of ammonium-fed plants on the K^+ concentration of the growth medium as compared to nitrate-fed plants (Lips et al., 1990).

6.5 CONCLUSIONS

The most significant findings of this research study are as follows.

(a) In hydroponically-grown maize, ammonium-fed plants show a significantly greater sensitivity to salinity than do nitrate-fed plants. This effect is most likely to occur under field conditions. These results suggest that by supplying nitrogen in the form of nitrate (instead of ammonium) to maize growing in saline soils, a maize farmer could minimize crop loss due to salinity toxicity.

(b) In nitrate-fed maize plants, inhibition of photosynthesis is not one of the manifestations of salinity toxicity. On the other hand the photosynthetic rates of ammonium-fed maize plants were significantly reduced by salinity. However, it is not clear whether this reduction in photosynthesis was caused by stomatal limitations, salinity ion toxicity or both.

(c) In both nitrate- and ammonium-fed plants the uptake and translocation of potassium in maize was significantly disturbed by salinity. ^{15}N studies carried out in this research also indicated salinity disturbance of the uptake and translocation of nitrogen in maize. In both nitrate- and ammonium-fed plants the moisture contents of whole plants, shoots and roots were significantly reduced by salinity. These findings are regarded as evidence for the supposition that induced nutrient deficiency and water stress are the major limitations on the growth of salt-stressed maize.

(d) When grown under low temperature conditions (25 ^{0}C) and supplied with lowest calcium concentration, salinity stressed maize grew significantly larger than non-salt-stressed plants, an effect which was not observed at high temperature (35 ^{0}C) conditions. These results indicate that when calcium supply in inadequate, low temperature promotes the stimulatory effect of NaCl on growth of maize whereas a high temperature promotes the inhibitory effect of NaCl on the growth of maize. This interaction between temperature and NaCl on the growth of maize is not well understood. The fact that the stimulatory effect of NaCl on maize grown at low temperature is mostly apparent when calcium supply is inadequate indicates a calcium sparing activity of NaCl.

(e) In all ranges of calcium concentrations (2.5 to 12 mM, 1 to 8 mM and 0.5 to 5 mM), no significant effect of calcium on photosynthetic rates and growth of salt-stressed maize was evident under high (35 ^{0}C) or low (25 ^{0}C) temperature conditions. These results indicate that the supposition that calcium plays a major role in enhancing salt tolerance in crop plants and that its ameliorative role could be temperature dependent is not true for this maize variety.

(f) Increasing the potassium concentration from 0.2 to 5 mM resulted in a significant increase in the growth of both non-salt-stressed and salt-stressed maize plants. This increment in growth could be ascribed to the role of potassium in nitrogen metabolism. Under non-saline conditions the growth of nitrate-fed maize plants did not significantly increase with an increase in potassium concentration from 2.5 to 5 mM. On the other hand an increase in potassium concentration from 2.5 to 5 mM resulted in a significant increase in the growth of ammonium-fed plants. These results indicate that the optimal concentration of potassium for ammonium-fed maize at 35 ^{0}C is higher than that used in conventional feeding solutions. This large requirement of K^+ for maximal growth of ammonium-fed maize plants could be ascribed to the absence of the K^+-shuttle in these plants.

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APPENDIX

APPENDIX 1. EQUATIONS USED FOR THE CALCULATION OF PHOTOSYNTHETIC RATE AND TRANSPIRATION RATE.

The internal microcomputer of the data processor (DL-1) of the LCA-2 infra-red gas analyser (Analytical Development Company, Hoddesdon, England) was programmed to compute net photosynthetic rate and transpiration rate using the following equations:

1.1. e_s = saturated vapour pressure at cuvette air temperature (units = bars)

$= 6.13753 \times \exp[t_a(18.564 - t_a/254.4)/(t_a + 255.57)] \times 10^{-3}$

where t_a represents cuvette air temperature

1.2. e_o = vapour pressure of water of air in cuvette (units = bars)

$= e_s \times h_c/100$

where h_c represents % relative humidity in cuvette

1.3. LH = latent heat of vaporization of water (units = J mol^{-1})

$= 2500 - (t_a \times 2.36)/18$

1.4. VF = mass flow of dry air per unit leaf area (units = mol m^{-2} s^{-1})

$= V \times 273/273+t_a \times 1/22.4 \times 1/1.013 \times 10/A$

where V represents flow rate of air supply unit in ml s^{-1} and

A represents projected leaf area in cm^2

1.5. H = radiation absorbed by the leaf (units = J m^{-2} s^{-1})

= (PFD x Ka) x (0.8 x 0.85 + 0.2 x 0.6)

where Ka = 698/3190 which converts μmol m^{-2} s^{-1} to W m^{-2}

0.8 = fraction of visible light absorbed by leaves

0.2 = fraction of infra-red light absorbed by leaves

0.85 = fraction of visible light transmitted through windows of leaf chamber

0.6 = fraction of infra-red light transmitted through windows of leaf chamber

PFD = photon flux density between 400 and 700 nm

therefore H = 0.175 x PFD

1.6. t = temperature difference between leaf and air

$= H - LH(E)/(0.93 \times Ma \times Cp/r_b + 4SB(t_a + 273)^3)$

where E = transpiration rate (refer to equation 8)

Ma = 28.97 (molecular weight of air)

Cp = 1.012 J g^{-1} K^{-1}

r_b = 0.144 m^2 s mol^{-1} (boundary layer resistance specified for leaf chamber

SB = 5.7 x 10^{-8} W m^{-2} K^{-4} (Stefan Boltzmann constant)

therefore t1 = leaf temperature

$= t_a - \Delta t$

1.7. e1 = saturated vapour pressure at leaf temperature (units = bars)

$= 6.1078 \times e^{(17.27 \times t1/t1 \times 237.3)}$

1.8. **E = transpiration rate (units = mmol m^{-2} s^{-1})**

$= (e_o/(P - e_o)) \times VF$

where P represents barometric pressure

1.9. C_c = analysis reading of cuvette carbon dioxide concentration corrected for analyser cross-sensitivity to water vapour

$= C_o - EMAX \times (1 + 7.87 \times 10^{-4} \times C_o) \times (1 - e^{(-0.07 \times e \times 1000)})$

1.10. **A = net photosynthetic rate (units = μmol m^{-2} s^{-1})**

$= (C_i - C_c/(P - e_o)) \times VF$

where C_i = reference carbon dioxide concentration measured by LCA-2 infra-red gas analyser.

APPENDIX 2. SUMMARY OF STATISTICS

Appendix table 2.1. A summary of t-tests which were performed to test if there were significant differences between the dry weights of whole plants, shoots, roots and shoot:root ratios of ammonium- or nitrate-fed maize grown in non-saline conditions and those of ammonium- or nitrate-fed maize grown in saline conditions.

	T-value	df	P-value
NITRATE-FED PLANTS			
Plant	4.656	62	< 0.05 **
Shoot	3.998	62	< 0.05 **
Root	5.559	62	< 0.05 **
Shoot:root ratio	1.939	62	> 0.05 NS
AMMONIUM-FED PLANTS			
Plant	9.925	62	< 0.05 **
Shoot	9.363	62	< 0.05 **
Root	6.744	62	< 0.05 **
Shoot:root ratio	0.538	62	> 0.05 NS

** = Significant
NS = Not significant

Appendix table 2.2. A summary of t-tests which were performed to test if there were significant differences between the dry weights of whole plants, shoots, roots and shoot:root ratio of salt-stressed or non-salt-stressed maize grown in nitrate and those of salt-stressed or non-salt-stressed maize grown in ammonium.

	T-value	df	P-value
NON-SALT-STRESSED PLANTS			
Plant	0.737	62	> 0.05 NS
Shoot	0.221	62	> 0.05 NS
Root	1.706	62	> 0.05 NS
Shoot:root ratio	2.479	62	> 0.05 NS
SALT-STRESSED PLANTS			
Plant	6.263	62	< 0.05 **
Shoot	5.781	62	< 0.05 **
Root	6.740	62	< 0.05 **
Shoot:root ratio	1.022	62	> 0.05 NS

** = Significant
NS = Not significant

Appendix table 2.3. A summary of t-tests which were performed to test if there were significant differences between the photosynthetic rates, transpiration rates and water use efficiencies of ammonium- or nitrate-fed maize grown in non-saline conditions and those of ammonium- or nitrate-fed maize grown in saline conditions.

	T-value	df	P-value
NITRATE-FED PLANTS			
Photosynthetic rate	1.219	18	> 0.05 NS
Transpiration rate	0.023	18	> 0.05 NS
Water use efficiency	1.599	18	> 0.05 NS
AMMONIUM-FED PLANTS			
Photosynthetic rate	7.292	18	< 0.05 **
Transpiration rate	6.955	18	< 0.05 **
Water use efficiency	2.355	18	< 0.05 **

** = Significant
NS = Not significant

Appendix table 2.4. A summary of t-tests which were performed to test if there were significant differences between the photosynthetic rates, transpiration rates and water use efficiency of salt-stressed or non-salt-stressed maize grown in nitrate and those of salt-stressed or non-salt-stressed maize grown in ammonium.

	T-value	df	P-value
NON-SALT-STRESSED PLANTS			
Photosynthetic rate	1.861	18	> 0.05 NS
Transpiration rate	2.047	18	> 0.05 NS
Water use efficiency	4.634	18	< 0.05 **
SALT-STRESSED PLANTS			
Photosynthetic rate	5.254	18	< 0.05 **
Transpiration rate	4.385	18	< 0.05 **
Water use efficiency	4.740	18	< 0.05 **

** = Significant
NS = Not significant

Appendix table 2.5. A summary of t-tests which were performed to test if there were significant differences between potassium contents of the shoots and roots of ammonium- or nitrate-fed maize grown in non-saline conditions and those of ammonium- or nitrate-fed maize grown in saline conditions.

	T-value	df	P-value
	NITRATE-FED PLANTS		
Shoot	2.694	14	< 0.05 **
Root	3.159	14	< 0.05 **
	AMMONIUM-FED PLANTS		
Shoot	5.363	14	< 0.05 **
Root	7.674	14	< 0.05 **

** = Significant

Appendix table 2.6. A summary of t-tests which were performed to test if there were significant differences between potassium contents of the shoots and roots of salt-stressed or non-salt-stressed maize grown in ammonium and those of salt-stressed or non-salt-stressed maize grown in nitrate.

	T-value	df	P-value
	NON-SALT-STRESSED PLANTS		
Shoot	8.428	14	< 0.05 **
Root	7.800	14	< 0.05 **
	SALT-STRESSED PLANTS		
Shoot	4.131	14	< 0.05 **
Root	6.740	14	< 0.05 **

** = Significant

Appendix table 2.7. **A summary of two-way and one-way Anovas for nitrate- and ammonium-fed maize plants grown in a range of calcium concentrations (2.5 to 12 mM) at 35 ^{0}C.** In both nitrate- and ammonium-fed plants two-way Anovas were performed to test for significant differences between the **dry weights of whole plants, shoots and roots** of non-salt-stressed plants and those of salt-stressed plants. One-way Anovas were performed to test for significant differences among various calcium treatments in both salt-stressed and non-salt-stressed plants.

			F-ratio	df	P-value
		NITRATE-FED PLANTS			
PLANT	Two-way Anova		118.88	1	< 0.05 **
	One-way Anova	No Salt	1.46	3	> 0.05 NS
		Salt-stressed	4.13	3	< 0.05 **
SHOOT	Two-way Anova		79.29	1	< 0.05 **
	One-way Anova	No Salt	1.63	3	> 0.05 NS
		Salt-stressed	3.14	3	< 0.05 **
ROOT	Two-way Anova		81.05	1	< 0.05 **
	One-way Anova	No Salt	1.53	3	> 0.05 NS
		Salt-stressed	4.69	3	< 0.05 **
		AMMONIUM-FED PLANTS			
PLANT	Two-way Anova		191.27	1	< 0.05 **
	One-way Anova	No Salt	0.28	3	> 0.05 NS
		Salt-stressed	2.75	3	< 0.05 **
SHOOT	Two-way Anova		160.71	1	< 0.05 **
	One-way Anova	No Salt	0.08	3	> 0.05 NS
		Salt-stressed	2.30	3	< 0.05 **
ROOT	Two-way Anova		171.10	1	< 0.05 **
	One-way Anova	No Salt	2.90	3	> 0.05 NS
		Salt-stressed	3.76	3	< 0.05 **

** = Significant
NS = Not significant

Appendix table 2.8. **A summary of two-way and one-way Anovas for nitrate- and ammonium-fed maize plants grown in a range of high calcium concentrations (2.5 to 12 mM) at 35 ^{0}C.** In both nitrate- and ammonium-fed plants two-way Anovas were performed to test for significant differences between the **moisture contents of whole plants, shoots and roots** of non-salt-stressed plants and those of salt-stressed plants. One-way Anovas were performed to test for significant differences among various calcium treatments in both salt-stressed and non-salt-stressed plants.

			F-ratio	df	P-value
		NITRATE-FED PLANTS			
PLANT	Two-way Anova		72.99	1	< 0.05 **
	One-way Anova	No Salt	2.23	3	> 0.05 NS
		Salt-stressed	9.89	3	> 0.05 NS
SHOOT	Two-way Anova		168.98	1	< 0.05 **
	One-way Anova	No Salt	7.039	3	> 0.05 NS
		Salt-stressed	10.85	3	> 0.05 NS
ROOT	Two-way Anova		12.72	1	< 0.05 **
	One-way Anova	No Salt	0.31	3	> 0.05 NS
		Salt-stressed	9.74	3	> 0.05 NS
		AMMONIUM-FED PLANTS			
PLANT	Two-way Anova		5.47	1	< 0.05 **
	One-way Anova	No Salt	5.56	3	> 0.05 NS
		Salt-stressed	1.81	3	> 0.05 NS
SHOOT	Two-way Anova		9.40	1	< 0.05 **
	One-way Anova	No Salt	7.54	3	> 0.05 NS
		Salt-stressed	0.84	3	> 0.05 NS
ROOT	Two-way Anova		117.00	1	< 0.05 **
	One-way Anova	No Salt	0.83	3	> 0.05 NS
		Salt-stressed	2.45	3	> 0.05 NS

** = Significant
NS = Not significant

Appendix table 2.9. **A summary of two-way and one-way Anovas for nitrate- and ammonium-fed maize plants grown in a range of calcium concentrations (2.5 to 12 mM) at 35 °C.** In both nitrate- and ammonium-fed plants two-way Anovas were performed to test for significant differences between the **photosynthetic rates and transpiration rates** of non-salt-stressed plants and those of salt-stressed plants. One-way Anovas were performed to test for significant differences among various calcium treatments in both salt-stressed and non-salt-stressed plants.

		F-ratio	df	P-value
	(a) **PHOTOSYNTHETIC RATE**			
	NITRATE-FED PLANTS			
Two-way Anova		0.17	1	> 0.05 NS
One-way Anova	No Salt	0.62	3	> 0.05 NS
	Salt-stressed	1.01	3	> 0.05 NS
	AMMONIUM-FED PLANTS			
Two-way Anova		2.85	1	< 0.05 **
One-way Anova	No Salt	2.42	3	> 0.05 NS
	Salt-stressed	0.56	3	> 0.05 NS
	(b) **TRANSPIRATION RATE**			
	NITRATE-FED PLANTS			
Two-way Anova		6.83	1	> 0.05 NS
One-way Anova	No Salt	3.82	3	> 0.05 NS
	Salt-stressed	1.15	3	> 0.05 NS
	AMMONIUM-FED PLANTS			
Two-way Anova		3.83	1	< 0.05 **
One-way Anova	No Salt	0.09	3	> 0.05 NS
	Salt-stressed	0.59	3	> 0.05 NS

** = Significant
NS = Not significant

Appendix table 2.10. **A summary of two-way Anovas and t-tests for nitrate-fed maize grown in a range of calcium concentrations (2.5 to 8 mM) at 35 °C.** Two-way Anovas were performed to test if there were significant differences between ^{15}N contents of shoots and roots of non-salt-stressed maize plants and those of salt-stressed plants. In both non-salt-stressed and salt-stressed maize plants t-tests were performed to test for significant differences between the two calcium treatments.

	F-ratio	df	P-value
Plant	10.39	1	< 0.05 **
Shoot	16.51	1	< 0.05 **
Root	4.48	1	< 0.05 **

** = Significant

	T-value	df	P-value
	NON-SALT-STRESSED PLANTS		
Plant	0.07	14	> 0.05 NS
Shoot	0.42	14	> 0.05 NS
Root	0.09	14	> 0.05 NS
	SALT-STRESSED PLANTS		
Plant	0.72	14	> 0.05 NS
Shoot	0.83	14	> 0.05 NS
Root	0.57	14	> 0.05 NS

NS = Not Significant

Appendix table 2.11. **A summary of two-way and one-way Anovas for nitrate-fed maize plants grown in a range of calcium concentrations (1 to 8 mM) at 35 ^{0}C and at 25 ^{0}C.** For both plants grown at 35 ^{0}C and at 25 ^{0}C two-way Anovas were performed to test for significant differences between the **dry weights of whole plants, shoots and roots** of non-salt-stressed plants and those of salt-stressed plants. One-way Anovas were performed to test for significant differences among various calcium treatments in both salt-stressed and non-salt-stressed plants.

			F-ratio	df	P-value
		PLANTS GROWN AT 35 ^{0}C			
PLANT	Two-way Anova		136.06	1	< 0.05 **
	One-way Anova	No Salt	3.17	3	< 0.05 **
		Salt-stressed	2.63	3	> 0.05 NS
SHOOT	Two-way Anova		128.36	1	< 0.05 **
	One-way Anova	No Salt	2.70	3	< 0.05 **
		Salt-stressed	3.29	3	> 0.05 NS
ROOT	Two-way Anova		117.76	1	< 0.05 **
	One-way Anova	No Salt	1.7	3	> 0.05 NS
		Salt-stressed	1.3	3	> 0.05 NS
		PLANTS GROWN AT 25 ^{0}C			
PLANT	Two-way Anova		0.32	1	> 0.05 NS
	One-way Anova	No Salt	23.85	3	< 0.05 **
		Salt-stressed	0.49	3	< 0.05 NS
SHOOT	Two-way Anova		0.86	1	> 0.05 NS
	One-way Anova	No Salt	29.88	3	< 0.05 **
		Salt-stressed	0.19	3	> 0.05 NS
ROOT	Two-way Anova		7.98	1	< 0.05 **
	One-way Anova	No Salt	10.89	3	< 0.05 **
		Salt-stressed	1.31	3	> 0.05 NS

** = Significant
NS = Not significant

Appendix table 2.12. **A summary of two-way and one-way Anovas for nitrate-fed maize plants grown in a range of low calcium concentrations (1 to 8 mM) at 35 °C and at 25 °C.** For both plants grown at 35 °C and at 25 °C two-way Anovas were performed to test for significant differences between the **moisture contents of whole plants, shoots and roots** of non-salt-stressed plants and those of salt-stressed plants. One-way Anovas were performed to test for significant differences among various calcium treatments in both salt-stressed and non-salt-stressed plants.

			F-ratio	df	P-value
		PLANTS GROWN AT 35 °C			
PLANT	Two-way Anova		28.30	1	< 0.05 **
	One-way Anova	No Salt	0.74	3	> 0.05 NS
		Salt-stressed	2.14	3	> 0.05 NS
SHOOT	Two-way Anova		28.48	1	< 0.05 **
	One-way Anova	No Salt	2.63	3	> 0.05 NS
		Salt-stressed	2.72	3	> 0.05 NS
ROOT	Two-way Anova		11.66	1	< 0.05 **
	One-way Anova	No Salt	0.14	3	> 0.05 NS
		Salt-stressed	1.51	3	> 0.05 NS
		PLANTS GROWN AT 25 °C			
PLANT	Two-way Anova		27.53	1	< 0.05 **
	One-way Anova	No Salt	1.14	3	> 0.05 NS
		Salt-stressed	1.67	3	> 0.05 NS
SHOOT	Two-way Anova		7.34	1	< 0.05 **
	One-way Anova	No Salt	5.46	3	> 0.05 NS
		Salt-stressed	0.86	3	> 0.05 NS
ROOT	Two-way Anova		0.44	1	< 0.05 **
	One-way Anova	No Salt	0.38	3	> 0.05 NS
		Salt-stressed	2.24	3	> 0.05 NS

** = Significant
NS = Not significant

Appendix table 2.13. **A summary of two-way and one-way Anovas for nitrate-fed maize plants grown in a range of calcium concentrations (1 to 8 mM) at 35 °C and at 25 °C.** For both plants grown at 35 °C and at 25 °C two-way Anovas were performed to test for significant differences between the **photosynthetic rates and transpiration rates** of non-salt-stressed plants and those of salt-stressed plants. One-way Anovas were performed to test for significant differences among various calcium treatments in both salt-stressed and non-salt-stressed plants.

		F-ratio	df	P-value
	(a) **PHOTOSYNTHETIC RATE**			
	PLANTS GROWN AT 35 °C			
Two-way Anova		1.49	1	> 0.05 NS
One-way Anova	No Salt	0.43	3	> 0.05 NS
	Salt-stressed	2.81	3	> 0.05 NS
	PLANTS GROWN AT 25 °C			
Two-way Anova		1.87	1	> 0.05 NS
One-way Anova	No Salt	2.53	3	> 0.05 NS
	Salt-stressed	0.75	3	> 0.05 NS
	(b) **TRANSPIRATION RATE**			
	PLANTS GROWN AT 35 °C			
Two-way Anova		0.22	1	> 0.05 NS
One-way Anova	No Salt	2.80	3	> 0.05 NS
	Salt-stressed	2.57	3	> 0.05 NS
	PLANTS GROWN AT 25 °C			
Two-way Anova		0.13	1	> 0.05 NS
One-way Anova	No Salt	1.44	3	> 0.05 NS
	Salt-stressed	0.38	3	> 0.05 NS

NS = Not significant

Appendix table 2.14. A summary of two-way Anovas which were performed to test for significant differences between the dry weights of whole plants, shoots and roots of salt-stressed and non-salt-stressed plants grown in a range of calcium concentrations (1 to 8 mM) at 35 ^{0}C and those of salt-stressed and non-salt-stressed plants grown in a similar range of calcium concentrations (1 to 8 mM) at 25 ^{0}C.

	F-ratio	df	P-value
NON-SALT-STRESSED PLANTS			
Plant	11.39	1	< 0.05 **
Shoot	18.51	1	< 0.05 **
Root	21.48	1	< 0.05 **
SALT-STRESSED PLANTS			
Plant	1.7	1	> 0.05 NS
Shoot	2.5	1	> 0.05 NS
Root	0.89	1	> 0.05 NS

** = Significant
NS = Not significant

Appendix table 2.15. A summary of two-way Anovas which were performed to test for significant differences between the moisture contents of whole plants, shoots and roots of salt-stressed and non-salt-stressed plants grown in a range of calcium concentrations (1 to 8 mM) at 35 ^{0}C and those of salt-stressed and non-salt-stressed plants grown in a similar range of calcium concentrations (1 to 8 mM) at 25 ^{0}C.

	F-ratio	df	P-value
NON-SALT-STRESSED PLANTS			
Plant	0.97	1	> 0.05 NS
Shoot	1.79	1	> 0.05 NS
Root	1.54	1	> 0.05 NS
SALT-STRESSED PLANTS			
Plant	1.7	1	> 0.05 NS
Shoot	2.5	1	> 0.05 NS
Root	0.89	1	> 0.05 NS

NS = Not significant

Appendix table 2.16. A summary of two-way Anovas which were performed to test for significant differences between the photosynthetic rates and transpiration rates of salt-stressed and non-salt-stressed plants grown in a range of calcium concentrations (1 to 8 mM) at 35 ^{0}C and those of salt-stressed and non-salt-stressed plants grown in a similar range of calcium concentrations (1 to 8 mM) at 25 ^{0}C.

	F-ratio	df	P-value
NON-SALT-STRESSED PLANTS			
Photosynthetic rate	16.39	1	< 0.05 **
Transpiration rate	15.15	1	< 0.05 **
SALT-STRESSED PLANTS			
Photosynthetic rate	12.78	1	< 0.05 **
Transpiration rate	17.67	1	< 0.05 **

** = Significant

Appendix table 2.17. **A summary of two-way and one-way Anovas for nitrate-fed maize plants grown in a range of calcium concentrations (0.5 to 5 mM) at 35 °C and at 25 °C.** For both plants grown at 35 °C and at 25 °C two-way Anovas were performed to test for significant differences between the **dry weights of whole plants, shoots and roots** of non-salt-stressed plants and those of salt-stressed plants. One-way Anovas were performed to test for significant differences among various calcium treatments in both salt-stressed and non-salt-stressed plants.

			F-ratio	df	P-value
		PLANTS GROWN AT 35 °C			
PLANT	Two-way Anova		11.32	1	< 0.05 **
	One-way Anova	No Salt	20.85	2	< 0.05 **
		Salt-stressed	0.49	2	> 0.05 NS
SHOOT	Two-way Anova		15.86	1	< 0.05 **
	One-way Anova	No Salt	23.88	2	< 0.05 **
		Salt-stressed	0.19	2	> 0.05 NS
ROOT	Two-way Anova		7.98	1	< 0.05 **
	One-way Anova	No Salt	10.89	2	< 0.05 **
		Salt-stressed	1.31	2	> 0.05 NS
		PLANTS GROWN AT 25 °C			
PLANT	Two-way Anova		9.39	1	< 0.05 **
	One-way Anova	No Salt	4.19	2	< 0.05 **
		Salt-stressed	0.29	2	> 0.05 NS
SHOOT	Two-way Anova		14.26	1	< 0.05 **
	One-way Anova	No Salt	2.87	2	> 0.05 NS
		Salt-stressed	0.02	2	> 0.05 NS
ROOT	Two-way Anova		1.71	1	> 0.05 NS
	One-way Anova	No Salt	4.81	2	< 0.05 **
		Salt-stressed	1.95	2	> 0.05 NS

** = Significant
NS = Not significant

Appendix table 2.18. **A summary of two-way and one-way Anovas for nitrate-fed maize plants grown in a range of calcium concentrations (0.5 to 5 mM) at 35 °C and at 25 °C.** For both plants grown at 35 °C and at 25 °C two-way Anovas were performed to test for significant differences between the **photosynthetic rates and transpiration rates** of non-salt-stressed plants and those of salt-stressed plants. One-way Anovas were performed to test for significant differences among various calcium treatments in both salt-stressed and non-salt-stressed plants.

		F-ratio	df	P-value
	(a) **PHOTOSYNTHETIC RATE**			
	PLANTS GROWN AT 35 °C			
Two-way Anova		0.97	1	> 0.05 NS
One-way Anova	No Salt	1.23	2	> 0.05 NS
	Salt-stressed	1.01	2	> 0.05 NS
	PLANTS GROWN AT 25 °C			
Two-way Anova		1.85	1	> 0.05 NS
One-way Anova	No Salt	0.76	2	> 0.05 NS
	Salt-stressed	0.56	2	> 0.05 NS
	(b) **TRANSPIRATION RATE**			
	PLANTS GROWN AT 35 °C			
Two-way Anova		1.83	1	> 0.05 NS
One-way Anova	No Salt	0.89	2	> 0.05 NS
	Salt-stressed	1.15	2	> 0.05 NS
	PLANTS GROWN AT 25 °C			
Two-way Anova		1.26	1	> 0.05 NS
One-way Anova	No Salt	0.49	2	> 0.05 NS
	Salt-stressed	0.59	2	> 0.05 NS

NS = Not significant

Appendix table 2.19. A summary of two-way Anovas which were performed to test for significant differences between the dry weights of whole plants, shoots and roots of salt-stressed and non-salt-stressed plants grown in a range of calcium concentrations (0.5 to 5 mM) at 35 °C and those of salt-stressed and non-salt-stressed plants grown in a similar range of calcium concentrations (0.5 to 5 mM) at 25 °C.

	F-ratio	df	P-value
NON-SALT-STRESSED PLANTS			
Plant	23.39	1	< 0.05 **
Shoot	19.15	1	< 0.05 **
Root	25.84	1	< 0.05 **
SALT-STRESSED PLANTS			
Plant	0.78	1	> 0.05 NS
Shoot	0.67	1	> 0.05 NS
Root	1.21	1	> 0.05 NS

** = Significant
NS = Not significant

Appendix table 2.20. A summary of two-way Anovas which were performed to test for significant differences between the photosynthetic rates and transpiration rates of salt-stressed and non-salt-stressed plants grown in a range of calcium concentrations (0.5 to 5 mM) at 35 °C and those of salt-stressed and non-salt-stressed plants grown in a similar range of calcium concentrations (0.5 to 5 mM) at 25 °C.

	F-ratio	df	P-value
NON-SALT-STRESSED PLANTS			
Photosynthetic rate	14.19	1	< 0.05 **
Transpiration rate	16.51	1	< 0.05 **
SALT-STRESSED PLANTS			
Photosynthetic rate	14.78	1	< 0.05 **
Transpiration rate	15.67	1	< 0.05 **

** = Significant

Appendix table 2.21. A summary of t-tests which were performed to test if there were significant differences between ^{15}N contents of the whole plants, shoots and roots of salt-stressed maize plants grown in lowest calcium concentration (0.5 mM) at 25 ^{0}C and those of salt-stressed plants grown under similar conditions.

	T-value	df	P-value
Plant	5.249	14	< 0.05 **
Shoot	3.226	14	< 0.05 **
Root	5.595	14	< 0.05 **

** = Significant

Appendix table 2.22. **A summary of two-way and one-way Anovas for nitrate- and ammonium-fed maize plants grown in a range of potassium concentrations (0.2 to 5 mM) at 35 ^{0}C.** In both nitrate- and ammonium-fed plants two-way Anovas were performed to test for significant differences between **dry weights of whole plants, shoots and roots** of non-salt-stressed plants and those of salt-stressed plants. One-way Anovas were performed to test for significant differences among various calcium treatments in both salt-stressed and non-salt-stressed plants.

			F-ratio	df	P-value
		NITRATE-FED PLANTS			
PLANT	Two-way Anova		263.08	1	< 0.05 **
	One-way Anova	No Salt	4.17	3	< 0.05 **
		Salt-stressed	5.53	3	< 0.05 **
SHOOT	Two-way Anova		256.81	1	< 0.05 **
	One-way Anova	No Salt	5.69	3	< 0.05 **
		Salt-stressed	3.58	3	< 0.05 **
ROOT	Two-way Anova		189.63	1	< 0.05 **
	One-way Anova	No Salt	1.13	3	> 0.05 NS
		Salt-stressed	1.39	3	< 0.05 **
		AMMONIUM-FED PLANTS			
PLANT	Two-way Anova		92.68	1	< 0.05 **
	One-way Anova	No Salt	24.86	3	< 0.05 **
		Salt-stressed	17.89	3	< 0.05 **
SHOOT	Two-way Anova		78.48	1	< 0.05 **
	One-way Anova	No Salt	25.65	3	< 0.05 **
		Salt-stressed	12.12	3	< 0.05 **
ROOT	Two-way Anova		102.65	1	< 0.05 **
	One-way Anova	No Salt	16.59	3	< 0.05 **
		Salt-stressed	14.19	3	< 0.05 **

** = Significant
NS = Not significant

Appendix table 2.23. **A summary of two-way and one-way Anovas for nitrate- and ammonium-fed maize plants grown in a range of potassium concentrations (0.2 to 5 mM) at 35 °C.** In both nitrate- and ammonium-fed plants two-way Anovas were performed to test for significant differences between **moisture contents of whole plants, shoots and roots** of non-salt-stressed plants and those of salt-stressed plants. One-way Anovas were performed to test for significant differences among various calcium treatments in both salt-stressed and non-salt-stressed plants.

			F-ratio	df	P-value
		NITRATE-FED PLANTS			
PLANT	Two-way Anova		47.84	1	< 0.05 **
	One-way Anova	No Salt	6.95	3	> 0.05 NS
		Salt-stressed	0.81	3	> 0.05 NS
SHOOT	Two-way Anova		46.85	1	< 0.05 **
	One-way Anova	No Salt	8.66	3	> 0.05 NS
		Salt-stressed	0.98	3	> 0.05 NS
ROOT	Two-way Anova		10.27	1	< 0.05 **
	One-way Anova	No Salt	3.25	3	> 0.05 NS
		Salt-stressed	2.01	3	> 0.05 NS
		AMMONIUM-FED PLANTS			
PLANT	Two-way Anova		126.09	1	< 0.05 **
	One-way Anova	No Salt	0.40	3	> 0.05 NS
		Salt-stressed	2.65	3	> 0.05 NS
SHOOT	Two-way Anova		65.34	1	< 0.05 **
	One-way Anova	No Salt	4.73	3	> 0.05 NS
		Salt-stressed	0.72	3	> 0.05 NS
ROOT	Two-way Anova		95.36	1	< 0.05 **
	One-way Anova	No Salt	2.98	3	> 0.05 NS
		Salt-stressed	4.09	3	> 0.05 NS

** = Significant
NS = Not significant

Appendix table 2.24. **A summary of two-way and one-way Anovas for ammonium-fed maize plants grown in a range of potassium concentrations (0.2 to 5 mM) at 35 ^{0}C.** Two-way Anovas were performed to test for significant differences between **photosynthetic rates and transpiration rates** of non-salt-stressed plants and those of salt-stressed plants. One-way Anovas were performed to test for significant differences among various calcium treatments in both salt-stressed and non-salt-stressed plants.

		F-ratio	df	P-value
	(a) PHOTOSYNTHETIC RATE			
Two-way Anova		36.30	1	< 0.05 **
One-way Anova	No Salt	2.04	3	> 0.05 NS
	Salt-stressed	0.48	3	> 0.05 NS
	(b) TRANSPIRATION RATE			
Two-way Anova		21.34	1	< 0.05 **
One-way Anova	No Salt	1.99	3	> 0.05 NS
	Salt-stressed	1.03	3	> 0.05 NS

** = Significant
NS = Not significant